PB-227 989

AEROBIC DECOMPOSITION OF ALGAE AND NUTRIENT REGENERATION

William J. Jewell, et al

Stanford University
Stanford, California

June 1968

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Department of Civil Engineering
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AEROBIC DECOMPOSITION OF ALGAE
AND
NUTRIENT REGENERATION

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Technical Report No. 91
June 1968

Federal Water Pollution Control Administration Research
Grant WP - 1037, September 1966 to June 1968

300

ABSTRACT

AEROBIC DECOMPOSITION OF ALGAE AND NUTRIENT REGENERATION

The incorporation of carbon into algal material results in production of oxygen and is accompanied by nitrogen and phosphorus uptake and synthesis. Subsequently, if the algae are decomposed under aerobic conditions, oxygen is absorbed while the synthesized particulate nitrogen and phosphorus are regenerated to soluble forms. Problems stemming from this usage of oxygen and the associated regeneration of nutrients are rapidly increasing due to man's influence on the eutrophication of natural waters. This study presents definitive hypotheses for natural aerobic algal decomposition and nutrient regeneration. Emphasis is placed on the significance of the refractory organic fraction of algae in the decay and nutrient regeneration processes. Other variables considered include: 1) water chemistry, 2) algal species, 3) decomposers (bacteria and animals), 4) age of algal culture, and 5) temperature.

Natural heterogeneous algal growth and decomposition were studied in a wide variety of natural waters, ranging from ocean water to mountain spring water. Also, pure algal cultures were studied in order to explain some of the complex processes occurring during algal growth and decay.

ALGAL YIELD

When nitrogen was the nutrient which limited algal growth, the maximum algal yield could be predicted from the following formula: $C = aN$; where C is the maximum quantity of organic mass (as COD) produced by the algae growing on a given quantity of available nitrogen, N, while "a" equalled approximately 100 with a maximum range of 50 to 155. When atmospheric nitrogen could be used by the culture through nitrogen fixation, "a" varied between 30 and 50 with a usual value of 50. The minimum phosphorus concentration in the algae for which the above expression was found to hold was 0.034 per cent of the total organic material (expressed as COD).

ALGAL DECOMPOSITION

A consideration of the various factors that influence algal decomposition led to the postulation of two separate rate equations to represent this process. When algae grow in a pure state and are subsequently seeded with decomposing bacteria and animals, decomposition can be empirically defined by a second order equation:

$$-dm/dt = K''m(m_1 - m)$$

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where m is the total biodegradable organic mass remaining at any time, m_1 is the total organic mass measured at time zero plus a constant to account for antecedent conditions and K" is a second order decay rate coefficient.

Another equation can be used for the more common case of algal decomposition, which occurs when the decomposer mass develops along with the algae. This is described by a first order equation:

$$-dm/dt = K'm$$

where K' is the first order decay rate coefficient, and the other symbols are as previously described.

Both decomposition equations require knowledge of the biodegradable fraction of algae and the decomposition rate coefficient. The biodegradable fraction is that portion which is susceptible to decomposition within a year's time and was found to vary with age in a defined manner between 30 and 70 per cent of the organic mass present before decomposition started. K' was found to vary with age in a well defined manner between 0.15 and 0.01 per day.

NITROGEN AND PHOSPHORUS REGENERATION

Of the total particulate N and P in algae, only a certain fraction is available for regeneration. Part of the remainder is held in the non-biodegradable or refractory material of the decomposers (N_{rd}, P_{rd}) and the rest in the refractory material of the algae (N_{ra}, P_{ra}). N_{rd} and P_{rd} were estimated from previous research. Two different cases were considered for the prediction of N_{ra} and P_{ra}. For young cells with N and P concentrations greater than or equal to 3 and 0.3 per cent respectively, N_{ra} and P_{ra} in the refractory algal material was found to be about 3.0 per cent for nitrogen and 0.3 per cent phosphorus.

For older cells N_{ra} and P_{ra} could be approximated by assuming that the N and P concentrations in the refractory fraction was equal to that in the total mass at the beginning of decomposition.

These estimates were found suitable with a nitrogen concentration ranging from 0.9 to 22 mg/l with phosphorus ranging from 0.3 to 1.0 mg/l.

It is believed that the potential maximum yield of algae, their subsequent aerobic decomposition, and the extent of nitrogen and phosphorus regeneration can be predicted from the hypotheses and empirical data presented.

TABLE OF CONTENTS

Page

I. INTRODUCTION 1

II. BACKGROUND 9

- A. The Decomposition of Algae 10
  - 1. Quantity of Algal Decomposition. 10
  - 2. Rate of Algal Decomposition. 12
- B. Factors Controlling the Decomposition of Algae 15
  - 1. Algae Controlled Decomposition Processes 16
  - 2. Effect of Bacteria and Animals on Algal Decomposition. . 18
- C. Nitrogen and Phosphorus Regeneration 28
  - 1. Quantity of Nitrogen and Phosphorus Regenerated. 29
  - 2. Rate of Nitrogen and Phosphorus Regeneration 32
- D. Factors Controlling Nitrogen and Phosphorus Regeneration . . 34
- E. Effect of Temperature on Algal Decomposition and Nutrient Regeneration 39
- F. Age As a Controlling Parameter in Algal Decomposition and Nutrient Regeneration. 41
- G. Summary. 44

III. THEORETICAL CONSIDERATIONS 46

- A. Algal Growth and Decomposition 46
  - 1. Algal Yield. 46
  - 2. Algal Decomposition. 54
    - a. Second Order Algal Decomposition 62
    - b. First Order Algal Decomposition. 64

TABLE OF CONTENTS (Cont'd.)

Page

B. Nitrogen and Phosphorus Regeneration 64
    1. Quantity of Nitrogen and Phosphorus Regenerated. . . 65
    2. Rate of Nitrogen and Phosphorus Regeneration 75

IV. EXPERIMENTAL PROCEDURES. 78
    A. Common Equipment and Experimental Conditions 80
    B. General Experimental Procedure 85
    C. Analytical Techniques. 87
        1. Suspended and Volatile Solids 88
        2. Total and Particulate COD. 90
        3. Organic Nitrogen 93
        4. Organic Nitrogen Content of Suspended Solids 94
        5. Ammonia Nitrogen Determination 94-B
        6. Nitrite Nitrogen Determination 94-C
        7. Nitrate Nitrogen Determination 94-C
        8. Nitrogen Balance 95
        9. Phosphorus 96
        10. Dissolved Oxygen 98
        11. Rate of Oxygen Uptake Determination. 99
        12. Organic Carbon 100
        13. Sample Preparation for Total Organic Carbon Test . . 101
        14. Filtrate Organic Carbon. 101
        15. Lipids 103
        16. Chlorophyll. 104
    D. Sources of Error 106

V. EXPERIMENTAL RESULTS 109
    A. Experiment I - High Nutrient Level Algal Growth and Decay. 109
    B. Experiment II - Treatment Plant Effluent Grown Algae . . 113
    C. Experiment III - Major Experiments on Natural Water Samples. 116

TABLE OF CONTENTS (Cont'd.)

Page

1. Growth of Algae. 121
2. Decomposition of Algae 135
3. Effect of Anoxic Conditions, Algal Cell Viability, and Light on the Decomposition of Algae. 151
4. Nitrogen and Phosphorus Regeneration 159

E. Experiment IV - Young Algal Culture Studies. 171
F. Experiment V - Pure Culture Study. 173
G. Experiment VI - Chlorophyll Study. 195
H. Experiment VII - Nutrient Supplementation Studies. . . . 201

VI. DISCUSSION OF RESULTS. 205

A. Algal Growth 205
1. Variation of Algal Composition 205
2. Potential Yield of Algae 207

B. The Decomposition of Algae 212
1. Second Order Decomposition Kinetics. 214
2. First Order Decomposition Kinetics 219
3. Effect of Temperature on Algal Decomposition 230
4. Effect of Light, Algal Cell Viability, Anoxic Conditions on the Decomposition of Algae 233
5. Mechanisms of Decomposition. 234

C. Correlation of Chlorophyll to Algal Mass 238
D. Regeneration of Nitrogen and Phosphorus. 239
1. The Effect of Algal Age and Refractory Fraction on the Regeneration Process 249
2. Effect of Temperature on Nutrient Regeneration . . . 251

VII. ENGINEERING SIGNIFICANCE 253

VIII. CONCLUSIONS. 262

IX. SUGGESTIONS FOR FUTURE WORK. 265

X. BIBLIOGRAPHY 266

TABLES

Number Page

1. Rate of oxygen utilization by algal systems in the dark seeded with bacteria and animals. 13

2. Respiration of three blue-green algae (27) 14

3. Summary of production of extracellular organic carbon by algae 17

4. Bacterial mass in ocean and lake waters 18

5. Assimilation of algae by various animals. 25

6. Effect of temperature on the oxygen uptake rates of various organisms and biological systems. 41

7. Average composition of actively growing algae and maximum variation in composition observed (per cent ash free dry weight). 53

8. Composition in per cent of organic portion of complex mixtures of bacteria and microscopic animals (activated sludge) fed different substrates(18). 67

9. Summary of experiments and objectives 79

10. Concentration of reagents used in the COD test. 90

11. Algal growth media used in Experiment I 109

12. Summary of the results of the decomposition of algae grown in media with relatively high nutrient concentrations (Experiment I). 111

13. Results from aerobic decomposition of algae grown in waste water treatment plant effluent. 115

14. Natural water samples studied 116

15. Initial composition of natural waters, and final adusted nitrate and phosphate concentrations. 118

16. Composition of natural synthetic river water. 119

17. Variation of particulate nitrogen and phosphorus in algal communities 132

TABLES (Cont'd.)

Number Page

18. Algal system composition before and after aerobic decomposition, Experiment III 143

19. Summary of the refractory organic fractions (f) of the simulated natural algal systems 145

20. Summary of initial decomposition rates based on the total organic mass (mg COD/mg SS-day) 146

21. Effect of anoxic conditions on the refractory organic fraction of algae 154

22. Change in algal lipid concentration during decomposition. 158

23. COD/N and COD/P at the beginning and end of decomposition and per cent regeneration in Experiment III 164

24. Summary of some of the nitrogen and phosphorus release rates in simulated natural algal communities (Exp. III) . 170

25. Refractory organic fraction of young concentrated algal cultures. 175

26. Algal system composition before and after aerobic decomposition, Experiment V. 179

27. Summary of the refractory fraction of seeded pure algal cultures. 183

28. Temperature effect on BOD_5 with pure and seeded algae . . 185

29. Summary maximum dissolved oxygen uptake rates (as determined from COD) for pure and seeded algal cultures (Experiment V). 186

30. Regeneration of nitrogen and phosphorus from decomposition of pure cultures of algae. 189

31. Nutrient regeneration with seeded pure cultures 193

32. Summary of some of the phosphorus and nitrogen release rates in pure and seeded algal systems. 194

33. Effect of added nutrients, organic material, and animals on 70 day old (sample 6) nutrient deficient algae over 20 days of decomposition. 202

TABLES (Cont'd.)

Number **Page**

34. Comparison of BOD_5 with standard BOD dilution water and deionized water as dilution water 203

35. Maximum variation of Nitrogen and phosphorus in algal communities 206

36. Summary of calculated first order decomposition rate coefficients (K') 220

37. Error in approximating the decomposition kinetics with first order equation in Experiment III. 223

LIST OF FIGURES

Number Page

1. Schematic illustration of algae-water interactions 3

2. Carbon cycle in a fresh water lake. 4

3. Nitrogen cycle in a fresh water lake. 5

4. Phosphorus cycle in a fresh water lake. 6

5. Typical nitrogen and phosphorus changes during the decomposition of phytoplankton in the dark. 30

6. The nitrogen composition of algal cells during growth on a given quantity of nutrients. 51

7. Illustration of the composition of algal systems as related to the biodegradability of algae at different stages of decompostion. 56

8. Natural algal degradation 59

9. Illustration of the two extreme cases of natural algal decomposition 61

10. Illustration of algal growth and decomposition apparatus. 81

11. Rate of inorganic carbon removal. 102

12. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations (Sample 1) 122

13. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations (Sample 2) 123

14. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations (Sample 3) 124

15. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations (Sample 4) 125

16. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations (Sample 5) 126

17. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations (Sample 6) 127

LIST OF FIGURES (Cont'd.)

Number Page

18. (A to F) Change of COD/N and COD/P ratios of suspended solids. 128

19. Correlation of production of extracellular organic carbon to algal growth 136

20. Change of total COD during simulated natural algal decomposition (Sample 1). 137

21. Change of total COD during simulated natural algal decomposition (Sample 2). 138

22. Change of total COD during simulated natural algal decomposition (Sample 3). 139

23. Change of total COD during simulated natural algal decomposition (Sample 4). 140

24. Change of total COD during simulated natural algal decomposition (Sample 5). 141

25. Change of total COD during simulated natural algal decomposition (Sample 6). 142

26. Change of refractory organic fraction with algal age. . . 148

27. Effect of reseeding and addition of nitrogen and phosphorus on the decomposition of refractory organic material of composite branch 6 of all natural algal systems (Experiment III). 149

28. Effect of algal system age on the quantity of refractory organic material formed.. 150

29. Comparison of the decomposition of live and disintegrated algal cells. 156

30. Change of particulate nitrogen and phosphorus as a result of nutrient regeneration during aerobic decomposition of sample 1, Experiment III. 160

31. Change of particulate nitrogen and phosphorus as a result of nutrient regeneration during aerobic decomposition of sample 6, Experiment III. 161

32. Effect of algal age on per cent particulate nitrogen and phosphorus regeneration during the decomposition of algae 165

LIST OF FIGURES (Cont'd.)

Number Page

33. Effect of initial algal nitrogen and phosphorus concentrations on the per cent regenerated during decomposition 166

34. Quantity of organic nitrogen and phosphorus held in refractory organic material of algal systems. 168

35. Summary of organic nitrogen and phosphorus held in refractory organic material of algal systems. 169

36. Pure algal growth 175

37. Correlation of extracellular organic carbon production with algal growth 177

38. The decomposition of pure and seeded pure cultures of *Chlorella pyrenoidosa* 180

39. The decomposition of pure and seeded pure cultures of *Chlamydomonas* cultures. 181

40. Effect of temperature on the refractory organic fraction of algal systems. 184

41. Change in particulate nitrogen and phosphorus during the decomposition of pure and seeded pure *Chlorella pyrenoidosa* cultures. 190

42. Change in particulate nitrogen and phosphorus during the decomposition of pure and seeded pure *Chlamydomonas* cultures. 191

43. Correlation of algal age with chlorophyll concentration, total mass, and oxygen uptake rate (*Chlorella*). 196

44. Correlation of algal age with chlorophyll concentration, total mass, and oxygen uptake rate (*Scenedesmus*). 197

45. Correlation of algal age with chlorophyll concentration, total mass, and oxygen uptake rate (*Lyngbya*). 198

46. Correlation of algal age with chlorophyll concentration, total mass, and oxygen uptake rate (Ulothrix plus unidentified algae) 199

LIST OF FIGURES (Cont'd.)

Number Page

47. Application of second order equation to data for young and old seeded Chlorella cultures 216

48. Effect of varying M_1 on the nature of second order algal decomposition 217

49. Effect of algal age on the first order rate of decomposition of simulated natural algal systems (Exp.III) . . 221

50. Comparison of predicted and measured values of nitrogen bound in the refractory organic material of simulated natural systems (Experiment III). 241

51. Comparison of predicted and measured values of phosphorus bound in the refractory organic material of simulated natural systems (Experiment III). 242

52. Comparison of predicted and measured values of nitrogen bound in the refractory organic material of seeded pure algal cultures (Experiment V). 243

53. Comparison of predicted and measured values of phosphorus bound in the refractory organic material of seeded pure algal cultures (Experiment V). 244

54. Comparison of calculated verses measured nitrogen and phosphorus regeneration 248

I. INTRODUCTION

Eutrophication, or the process of enrichment of a water body by nutrients (1), causes an initially clear water body to become filled with organic and inorganic debris over its lifetime. Processes such as precipitation and drainage continue in the absence of man and usually result in a relatively slow enrichment process. The effects of man's activities through alteration of the landscape for agricultural and industrial expansion and the discharge of large quantities of domestic and industrial wastes in many instances has increased the natural rate of enrichment of waters many fold. One major result of increased eutrophication is the increase in production of phytoplankton and littoral vegetation. The generally undesirable situations that have been noted to occur with increased phytoplankton production have been collectively termed "algal problems" or "nutrient associated problems" (2,3,4,5). These may range from objectionable taste and odors or other violations of aesthetic senses, physical clogging of water supply systems, or changes in concentration or form of oxygen or other chemicals in a water body. It has been reported that all of the man-made water bodies (1) and 56 per cent of the United States surface water supplies are presently plagued by some type of algal associated problem (5). There is great cause for concern since the man-made rate of enrichment of water bodies is expected to double by the year 2000 (6).

In an attempt to define some of the mechanisms which control algal growth and decay, major emphasis in this study was placed on the relationship between nitrogen and phosphorus and algal growth, and the release of these elements and the consumption of oxygen during aerobic algal decomposition. The processes which were studied and the terms used to define these processes are illustrated in Figure 1. During algal growth, nitrogen and phosphorus are absorbed from solution and incorporated into algal cellular material. The synthesis of soluble carbon, nitrogen and phosphorus may be termed element incorporation. After an algal cell is formed, numerous factors may cause the release back to solution of the absorbed elements in an organic or an inorganic form. The release of carbon dioxide and soluble organic carbon can be referred to as regenerated carbon and similar terms may be used for the release of extracellular organic or inorganic nitrogen and phosphorus. Note that nutrients which are undergoing active turnover in the cell and between cells are not present in solution for a significant period of time and thus are not considered to be regenerated nutrients in this study.

Some of the natural biological reactions which carbon, nitrogen, and phosphorus may be involved with in natural waters are shown in Figures 2 to 4 which give expanded carbon, nitrogen, and phosphorus cycles taken from Figure 1 for greater detail. A complete definition of the fate of these elements was beyond the scope of this study. The reactions which this study considered are enclosed by dotted lines in these figures. In each case the processes studied were defined by attempting to answer three questions relating to: 1) the quantity of material involved in the reaction or change from one form to another, 2) the rate at which the reaction takes place, and 3) the mechanism

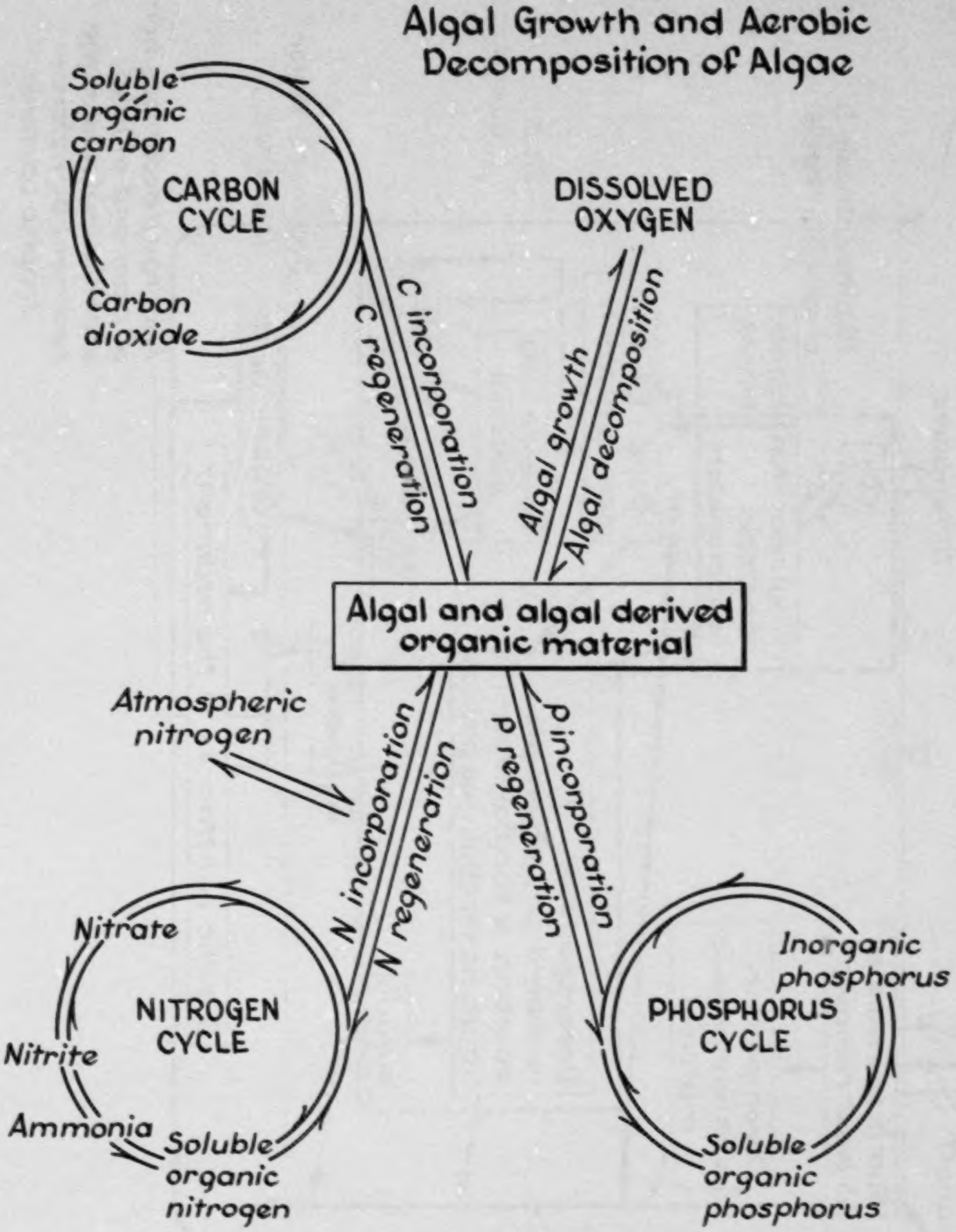
Algal Growth and Aerobic Decomposition of Algae
Soluble organic carbon
CARBON CYCLE
Carbon dioxide
DISSOLVED OXYGEN
C incorporation
C regeneration
Algal growth
Algal decomposition
Algal and algal derived organic material
Atmospheric nitrogen
N incorporation
N regeneration
P incorporation
P regeneration
Nitrate
Nitrite
Ammonia
NITROGEN CYCLE
Soluble organic nitrogen
Inorganic phosphorus
PHOSPHORUS CYCLE
Soluble organic phosphorus

FIGURE 1. Schematic illustration of Algae-Water Interactions. Algal growth is accompanied by carbon, nitrogen, and phosphorus incorporation; and aerobic decomposition of algae is accompanied by carbon, nitrogen, and phosphorus regeneration.

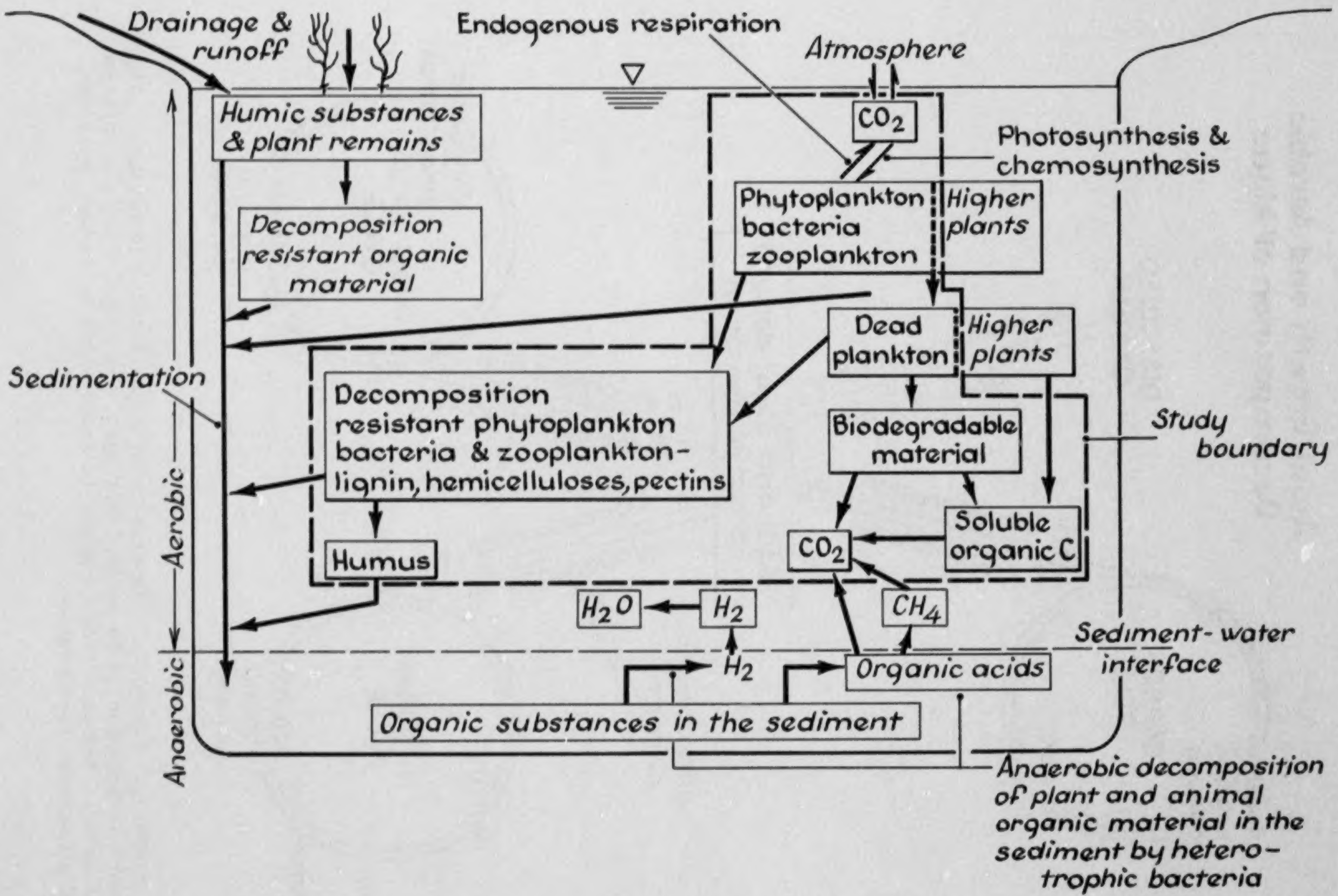
Drainage & runoff
Endogenous respiration
Atmosphere
CO_2
Photosynthesis & chemosynthesis
Humic substances & plant remains
Decomposition resistant organic material
Phytoplankton bacteria zooplankton
Higher plants
Dead plankton
Higher plants
Sedimentation
Decomposition resistant phytoplankton bacteria & zooplankton-lignin, hemicelluloses, pectins
Biodegradable material
Study boundary
Soluble organic C
CO_2
Humus
Aerobic
H_2O
H_2
CH_4
Sediment-water interface
Anaerobic
H_2
Organic acids
Organic substances in the sediment
Anaerobic decomposition of plant and animal organic material in the sediment by hetero-trophic bacteria

FIGURE 2. Carbon cycle in a fresh water lake. Dotted lines indicate factors considered in this study.

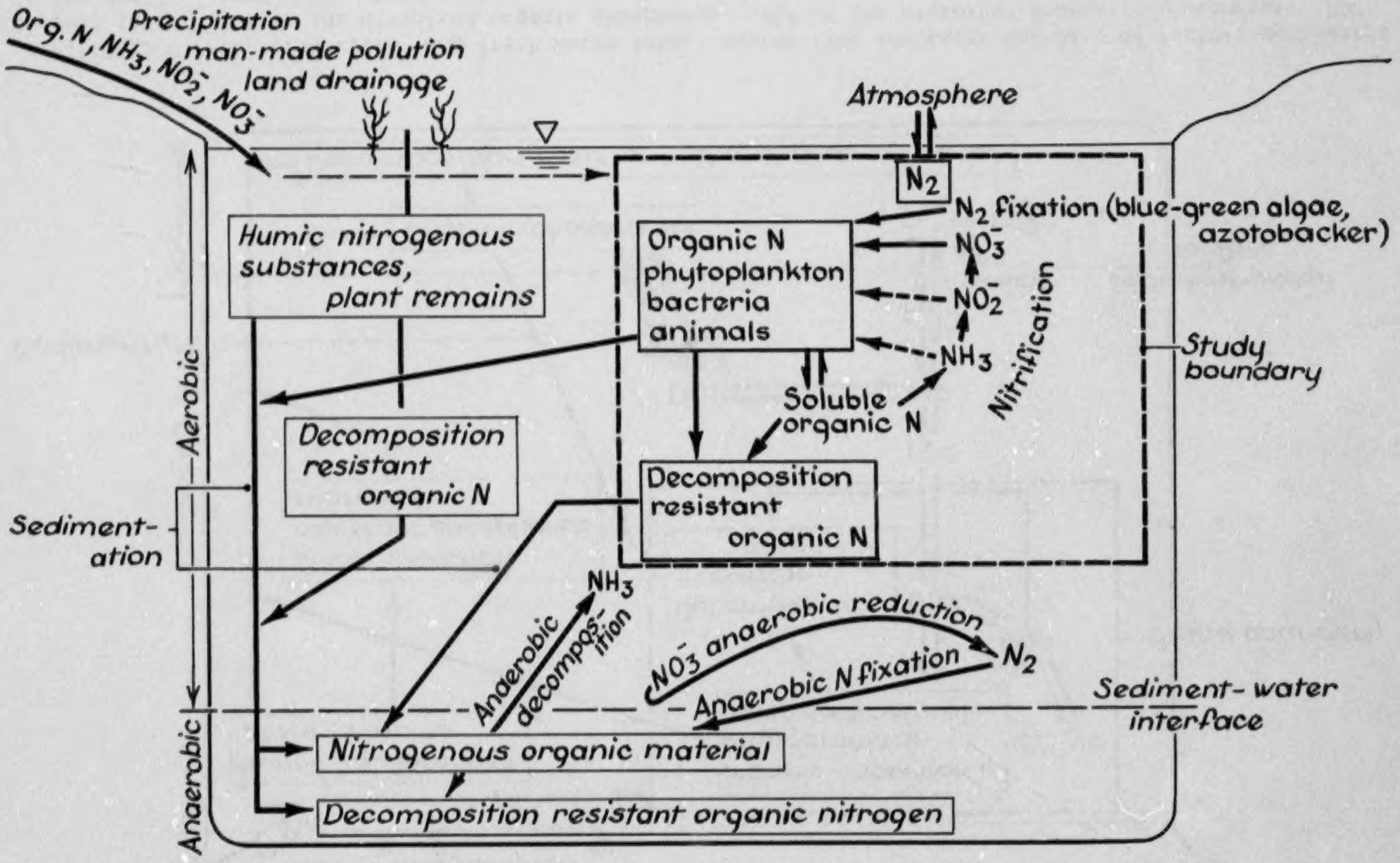
Org. N, NH_3, NO_2^-, NO_3^-
Precipitation
man-made pollution
land drainage
Atmosphere
N_2
N_2 fixation (blue-green algae, azotobacker)
NO_3^-
NO_2^-
NH_3
Nitrification
Study boundary
Humic nitrogenous substances, plant remains
Organic N phytoplankton bacteria animals
Soluble organic N
Decomposition resistant organic N
Decomposition resistant organic N
Aerobic
Sediment-ation
Anaerobic
NH_3
Anaerobic decompos-ition
NO_3^- anaerobic reduction
N_2
Anaerobic N fixation
Sediment-water interface
Nitrogenous organic material
Decomposition resistant organic nitrogen

FIGURE 3. Nitrogen cycle in a fresh water lake. Dotted line indicates factors considered in this study.

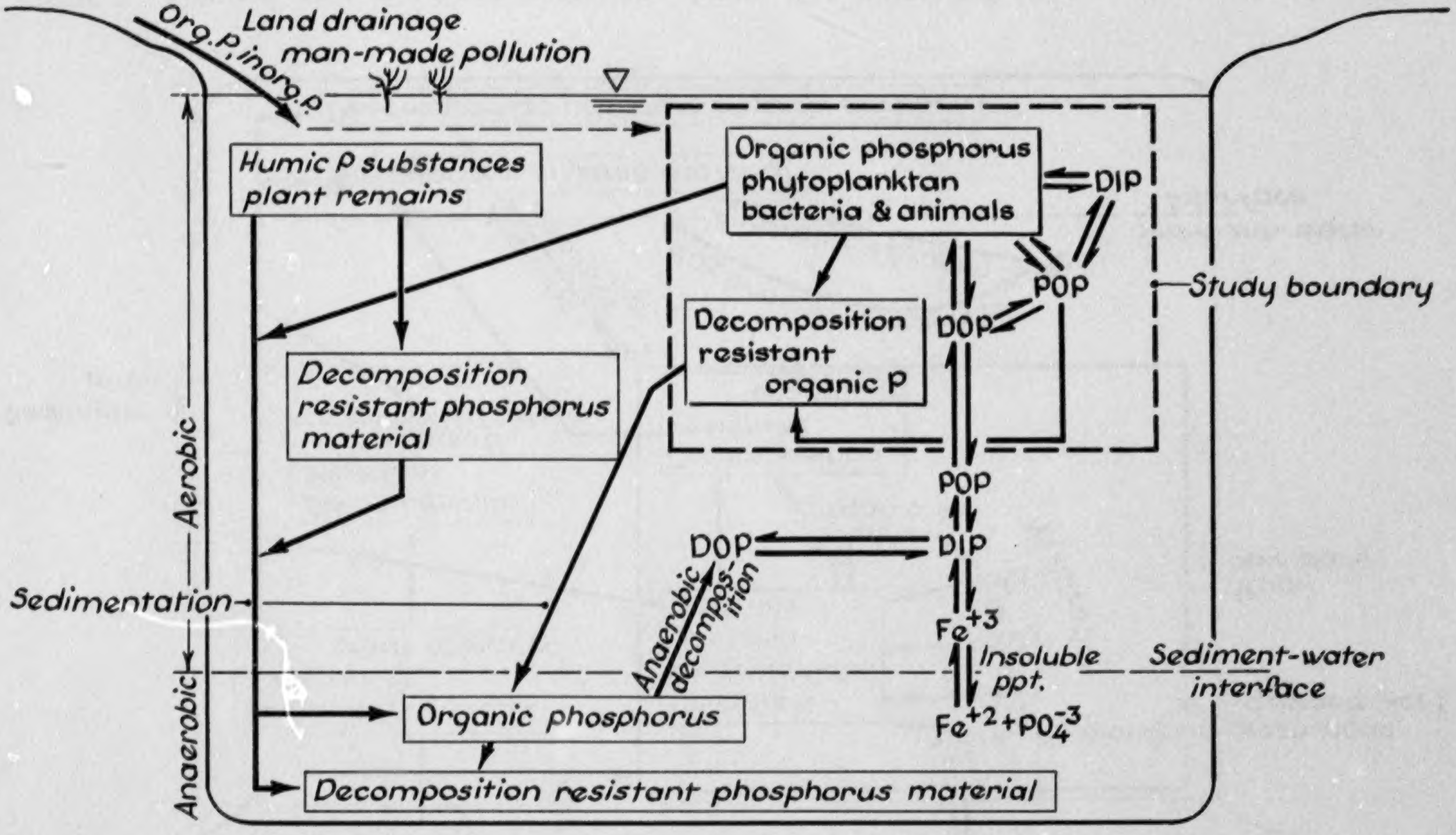
Land drainage
man-made pollution
Org. P, inorg. P
Humic P substances plant remains
Decomposition resistant phosphorus material
Organic phosphorus phytoplanktan bacteria & animals
DIP
POP
DOP
Decomposition resistant organic P
Study boundary
POP
DIP
DOP
Anaerobic decomposition
Fe^{+3}
Insoluble ppt.
$Fe^{+2}+PO_4^{-3}$
Sediment-water interface
Sedimentation
Aerobic
Anaerobic
Organic phosphorus
Decomposition resistant phosphorus material

FIGURE 4. Phosphorus cycle in a fresh water lake. Dotted line indicates boundary of factors considered in this study. DOP is the dissolved organic phosphorus. DIP is the dissolved inorganic phosphorus. POP is the particulate organic phosphorus.

which causes the reaction to occur. In general there were three questions of quantitative significance: 1) What quantity of algae can grow in a natural water; 2) What quantity of the algae that grows remains as humus after biodegradation (as shown in Figure 2); and 3) What quantity of the nitrogen and phosphorus remains in the resistant material which is left after biodegradation is complete?

Just as important as determining the quantity of material involved in a reaction is the rate of the reaction. The general questions studied and related to this aspect can be summarized as follows: 1) How fast are algae removed from water by the aerobic decomposition process, and 2) How fast are the particulate nitrogen and phosphorus regenerated? Finally, the questions relating to the mechanisms controlling the reactions considered may be summarized as: 1) What factors control the decomposition process, i.e., what organisms and conditions are responsible for carbon regeneration, and 2) What factors control nitrogen and phosphorus regeneration? The investigation of mechanisms was limited to organism relationships rather than molecular relationships, and most of the comments are confined to this level.

This study was designed to investigate the above questions using both an ecological approach employing mixed cultures of algae, bacteria, and animals; and a completely defined pure culture approach. Natural heterogeneous algal growth and decomposition were studied in water collected from sources ranging from the ocean to a mountain stream. In addition, pure algal cultures were used along with a completely defined "synthetic river water."

The following were the general objectives of this study:

1. To determine if any generally applicable relationships could be found regarding algal growth and decomposition in natural waters.
2. To evaluate the significance of algal decomposition on the oxygen, nitrogen, and phosphorus resources of natural waters.
3. To determine the relative effect on decomposition of factors such as: 1) anoxic conditions; 2) viability of the algae; 3) short periods of illumination; 4) algal system age; 5) predation by bacteria and animals; and 6) temperature.
4. To explain the mechanisms of aerobic decomposition of algal communities, and nitrogen and phosphorus regeneration.
5. To formulate the results into relationships which will enable one to predict the rate and extent of aerobic algal decomposition and nutrient regeneration in natural waters.

II. BACKGROUND

The stoichiometry of the growth and aerobic decomposition of algae may be represented by the following expression:

$$aCO_2 + cNO_3^- + ePO_4^{-3} + (c+3e)H^+ + 1/2(b-c-3e)H_2O \underset{\text{AEROBIC DECOMPOSITION}}{\overset{\text{GROWTH}}{\rightleftharpoons}} C_aH_bN_cO_dP_e + (a + b/4 + 5c/4-d/2 + 5e/4)O_2 \qquad \text{Eq (1)}$$

In this equation a, b, c, d, and e vary depending on the species and age of the algae, the available nutrients, temperature, and other related factors.

As algae grow they absorb nitrogen, phosphorus, and carbon; and release oxygen. The beneficial aspects of the production of oxygen during algal growth have been emphasized (7,8,9,10). However, little regard has been given to the use of oxygen and the release of nutrients during aerobic decomposition of algae (11,12), as given by the reversal of Equation 1.

As shown in Figure 1, regeneration in this study refers to the release of material to solution from the particulate matter. Regenerated carbon, nitrogen, or phosphorus may then refer to any form of these materials released and present in solution. The quantity of oxygen used in the oxidation of the organic matter will be related to the decomposition of algal organic material. The net quantity of nitrogen and phosphorus irreversibly released during this process will be referred to as regenerated nutrients.

The summary of information obtained from the literature pertaining to the decomposition of algae presented in the following paragraphs will be divided into two general categories: 1) Algal organic material decomposition; and 2) Nitrogen and phosphorus regeneration. Each of these categories will be discussed in relation to the quantity of material involved in the reaction, the speed at which it takes place, and the mechanisms involved.

THE DECOMPOSITION OF ALGAE

Quantity of Algae Decomposed

The quantity of algae which is susceptible to biological decomposition and the factors that affect this quantity have been of much concern to engineers faced with problems related to the presence of algae in natural waters. Some researchers believe that algae are one hundred per cent biodegradable (i.e., Equation 1 is completely reversible)(13,14). This philosophy is based on the hypothesis of "microbial infallibility," which can be traced as far back as Beijerinck (but not attributed to him)(15). The basic principle of microbial infallibility is that either by mutation and natural selection or by a nongenetic adaptation, one or another species appears always to develop in response to a modification in the habitat or by the appearance of new types of carboneceous nutrients. By this adaptation it is felt by supporters of the hypothesis that every biologically synthesized organic molecule will, under some set of circumstances, be destroyed by one or more species.

Alexander refers to the hypothesis of "microbial infallibility" as one of the "grand illusions of microbiology" (15). The failure of microorganisms to complete their task according to the infallibility hypothesis is brought about by two general causes: 1) a particular environment, microenvironment, or local conditions may not be conducive to microbial life or to the degradation of a specific compound; and 2) the existence of substances which are resistant either totally or partially to biodegradation.

Other researchers concerned with the biodegradability of algae have assumed that algal organic material is similar to bacterial and microscopic animal organic material (10,16) and therefore will have a similar proportion available for biological decomposition. The portion of newly synthesized bacterial and microscopic animal organic material which is not readily biologically decomposable has been found to vary between 20 and 25% (17,18).

Several studies have had as their major objective the determination of the biodegradable fraction of algal material (19,20,21,22). Two of these studies showed that under anaerobic conditions (up to two years of continuous digester operation, detention times of up to 60 days and temperature of up to 50°C) algae (predominantly Scenedesmus) had a surprisingly high nonbiodegradable fraction varying between 51 and 76% in all studies. Golueke et al.(21) suggested two possible reasons for this: 1) the algae remained alive and had some unknown defense against bacterial attack; or 2) the algal cell walls may have been highly resistant to bacterial attack. However, little supporting evidence was presented to prove either of these suggestions.

In a study of the aerobic decomposition of algae from a reservoir it was found that after the decomposition processes had ceased (over a period of 60 days) 33% of the initial total organic material was still present (20). No explanation for the occurrence of this resistant organic material was given. Thus, the portion of algal material which is resistant to biological decomposition appears quite high.

Rate of Algal Decomposition

The rate at which aerobic decomposition of algal material proceeds can be determined by measuring the rate of oxygen usage by decomposing algae. Such rates have been expressed by using a first order rate coefficient ($K_{base\ e} = mgO_2$ used per mg suspended solids per day). Typical slowly biologically oxidizable material have K rates in the order of 0.01 and readily oxidizable material have K rates of 0.20 or higher. Table 1 summarizes some of the rates of oxygen usage obtained for various algal systems. The oxygen utilization rates in this table include both the oxygen usage of the decomposers which consume the algae and the algal endogenous respiration. These rates differ from the reported respiration rates of pure algae in that the rates of respiration of pure cultures are usually measured over a period of minutes or hours. The reason for this short period of measurement is because of the rapidly changing respiration rate in pure algal cultures. Cramer and Myers (23). have shown that with Chlorella exposed to the light with excess inorganic nutrients in solution that a high rate of respiration is initially observed when the cells are initially placed in the dark (0.17 $mgO_2/mgSS/day$). This high rate may drop 75 per cent in the first four and a half hours. In Cramer and Myers study at the end of 10 to 12 hours the respiration rate had decreased to 0.077 $mgO_2/mgSS/day$. The rate continued to fall until it reached a constant value of 0.045 after 12 hours. They (23) showed that a carbohydrate storage product measured as starch was being utilized in the first 10 hours and that all starch disappeared after this time, thus correlating with the time of maximum rate decrease.

Table 1. Rate of oxygen utilization by algal systems in the dark seeded with bacteria and animals

ALGAE OR ALGAL SYSTEM	CONDITIONS	UTILIZATION RATE (mg O_2 per mg ss per day)	TEMP. (^{o}C)	REFERENCE
Chlorella pyrenoidosa	Culture age varying from 3 to 24 days; pH varying from 6.0 to 9.1; culture media - Gorham's, Myer's, Allen's; 7 fold seed volume and 10 fold oxygen depletion during the BOD test	Maximum range= .012 to .062 Average range= .018 to .04	23	24
Microcystis aeruginosa	Culture age varying from 3 to 24 days	Maximum range= .015 to .026	23	2
Scenedesmus dimorpha	Young cells grown in Allen's media	.026	23	2
Nostoc muscorum	Young cells	.016	23	2
Gloeotrichia echinulata	Cultures grown in Gorham's media, Gorham's media minus nitrogen, and Allen's media	Range = .01 to .026 Average = .016	23	2
Chlorella	17 different dilution waters used in the BOD test varying from lake water, deionized water, to supplemented standard dilution water	Maximum range= .01 to .084 Average range= .014 to .05	23	25
Natural river algae	Growing in Fox River, Wisconsin. 35 different samples	.04 to .07*	20	11
Natural reservoir algae	Algae growing in Russian reservoirs	Average = .037*	16	20

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*Rate refers to BOD K (base e) rate, all other above values calculated on basis of oxygen used in 5 days divided by the initially measured suspended solids.

These authors concluded that the rate of 0.047 is more a measure of the endogenous respiration rate than was the initial rate. Cramer and Myers (23) also estimated that this level of respiration was maintained for several days. Thus it can be seen that if the initial rate of respiration is 0.17 mgO_2/mgSS/day for 10 hours and then drops to 0.05 mgO_2/mgSS/day for five more days the total quantity of cell material utilized in respiration represents 26 per cent of the total cell (assuming 1.42 mg COD equals 1 mg VSS). Gibbs (26) has reported the initial autotrophically growing respiration rate of Chlorella pyrenoidosa as varying between 0.034 and 0.048. Kratz and Myers (27) have reported the following respiration rates for pure cultures of blue-green algae as measured upon removal from actively growing conditions and after 24 hours in the dark.

Table 2. Respiration of Three Blue-Green Algae (27).

ALGAE	TEMP.(°C)	CONDITION FOR CELLS	UTILIZATION RATE (mgO_2/mgSS/day)
Anabaena variabilis	25	Actively growing autotrophically	.29
		After 14 hours in dark	.06
Anacystis nidulans	25	Actively growing autotrophically	.05
		After 24 hours in dark	.01
Nostoc muscorum	25	Actively growing autotrophically	.15
		After 24 hours in dark	.03

Note that the oxygen utilization rate decreases nearly five fold in 24 hours in all algae in Table 2. It should also be noted that when nitrogen was missing from the medium the respiration rate fell much faster when algal cells were taken from the light and placed in the dark (23). Thus although prior conditions have a large effect on the respiration of algal cells it can be seen that endogenous respiration probably will not account for the decomposition of more than 20 to 30 per cent of the total cellular material in most algae.

When oxygen utilization by the algae and the decomposers is measured in the dark over a period of five days or more, the initial high oxygen utilization rates are masked. This is apparent in the data shown in Table 1. The important point to note in this table is that even with the presence of different decomposers, with various algal genera, at different ages, and at different temperatures, the oxygen utilization rates have a maximum range for all studies reported varying between 0.01 and 0.084, with most values falling between 0.01 and 0.04. Thus in all studies carried out over a time period of up to 25 days with live intact algae exposed to bacteria and microscopic animals, the rates of decomposition were characteristic of slowly oxidizable organic material.

FACTORS CONTROLLING THE DECOMPOSITION OF ALGAE

Fitzgerald (24) showed that addition of up to 30 milliliters per liter of settled domestic sewage had little effect on the BOD of algae and also had little effect on their oxygen producing potential. However, the supernatant liquor from algal cultures seeded with bacteria had up to a five fold smaller BOD than the supernatant from pure algal cultures. These two observations would indicate the decomposers were living on extracellular algal products in these tests. He then killed the algal cells by freeze-drying methods and found that these cells had a BOD_5 approximately four fold higher than that of living cells. Others (11,12, 28) have also determined the effect on the BOD test of killing algal cells by various methods and in all but a few instances reported a large increase in the oxygen usage over a period of five days as compared to live intact algae. Although these results indicate quite clearly the fact that the hydrolysis of soluble algal material to a soluble substrate is the controlling factor in algal decomposition no explanation of the controlling factors has been presented.

Algae Controlled Decomposition Processes

There are two general mechanisms which control the regeneration of carbon (as defined in Figure 1) from algae. First, the algal cell is self decomposed by lysing and endogenous respiration. Secondly, the algal cell is attacked by decomposing agents such as bacteria and animals. The maximum amount of cellular material used in endogenous respiration by the algae probably rarely amounts to more than 20% of the total cell material in young cells and much less than this in old cells (see earlier comments and Table 2). Therefore, it can be concluded that autoregeneration of carbon by endogenous respiration is a minor consideration in the overall decomposition process. Initially high oxygen uptake rates caused by rapid depletion of carbohydrate stores (23) or absorption of nitrogen (29,30) have been reported to cause high short term rates but these rates will have little significance in the overall algal decomposition process.

Another mechanism by which algal cell material may be decreased is by the excretion of soluble organic extracellular products. Note that the excretion of soluble organic material from algae is usually differentiated from lysing (which occurs with bacteria) since the former is observed with live and intact cells. The production of extracellular organic material and the quantity that may be expected with many genera of algae is indicated in Table 3.

Table 3. Summary of Production of Extracellular Organic Carbon by Algae

ALGAE	CONDITIONS	% TOTAL ORGANIC MATTER EXCRETED INTO SOLUTION	REFERENCE
Green Algae	young old	2 12.5	30
24 Species Natural Algae	During growth	average 2	31
Natural field studies	Lakes and Seas	7 - 50	33
22 Species Marine Algae	Laboratory Field studies	3-6 12	32
Several species Chlamydomonas	Actively growing	10-45	34

It has been noted in laboratory studies that for nearly all algae, excretion of soluble organic matter accounts for around 3% of the total organic matter in the system (30). Several authors have noted that the amount of extracellular organic material produced by the algae in its natural surroundings was much more than that found in pure cultures in the laboratory (31,32,33). The reason for this difference is not clear. However, in most cases it would not be expected that more than 10% of the total organic material would be released to solution. One notable exception is that given by the Chlamydomonas species (35). In these species there is an outer capsule containing polysaccharides (200 to 660Å thick) appearing as a fibrillose felt with a frayed outer surface. As the cell grows, this capsular material continually diffuses into the medium. Allen (34) studied several different species of Chlamydomonas and concluded that a relatively large amount of excretion is a general characteristic of this genera, and varied between 10 and 45% of the total organic matter found for actively growing cells (7 days old).

Thus the regeneration of carbon by forces within the algal cell probably amounts to less than 30% of the total organic matter fixed by algal growth. Only in special instances, such as with species of *Chlamydomonas*, does it appear that regeneration of carbon by endogenous respiration or excretion would result in more than 50% reduction of the total particulate organic matter in an aerobic algal system.

Effect of Bacteria and Animals on Algal Decomposition

The second general mechanism for carbon regeneration results from the destruction or uptake of algal material by other living organisms while using algal cells as food. In general, all these organisms may be called the algal decomposers. During decomposition a portion of the algal carbon is incorporated into the cells of the decomposers. This fixation by the decomposers may be called secondary productivity (36).

Bacteria-Algae Interactions

The decomposers may be classified in two categories: 1) bacteria, and 2) animals. Bacteria in natural water have been the object of many studies and a brief review of this area shows that bacteria are present in relatively large numbers in all oceans and fresh waters. Typical numbers are shown in Table 4.

Table 4. Bacterial Mass in Ocean and Lake Waters.

AREA	RANGE OF BACTERIA		REFERENCE
	MASS (mg/m^3)	NUMBERS (Thousands of cells per ml)	
Pacific Ocean	.008-25	0.04-125	37
Caspian Sea	0.20 -44.8	1 -224	37
North Pole	0.03 - 1.8	0.15- 9	37
Black Sea	7.70 -43.1	38 -167	37
529 Wisconsin Lakes	2.3 -244	19 -2050	38

It has been estimated that bacteria comprise about 0.1 to 1 per cent of the organic matter (by weight) in all lakes and oceans where algae form the main source of energy (35,39,40,41,42). The fact that bacteria form such a low fraction of the total organic mass and that this mass is always a relatively constant proportion of the total organic mass for a wide variety of conditions ranging from laboratory mass culture studies (39) to natural field conditions (41,42) is of significance in explaining the mechanism of algal decomposition and will be further emphasized in the theoretical considerations section.

Bacterial associations with algae and the possible mechanisms used in bacterial attack on the algae represent one of the most important but least researched areas of study (43,44). Ward *et al*. (39) studied the association of bacteria with a mass culture of one species of *Chlorella*. By obtaining samples of one species being studied in several different areas of the United States the authors were able to determine whether any specificity existed among the bacteria and what their effect on the growth of the algae must be. The results showed that several bacterial species were common to all samples and that their presence decreased the yield of algae by approximately 7% in 24 hours when compared with sterile controls. Each bacterial species tested by itself with the algae caused about the same amount of change in the algal yield and the same amount of algal growth yield decrease was recorded with all the bacteria present. A close correlation between algal growth and bacterial growth was found in this study. In all cases the bacteria were found to represent less than 1% of the total dry weight of the cultures. The authors showed that bacterial growth in these experiments was limited by the excretion rate of organics from the algae and no evidence was found of bacteria attacking or affecting the intact algal cell. None of the bacteria isolated were known to metabolize cellulose.

All evidence to date indicates that live algae possess an innate resistance to bacterial degradation (43,44,45). In the only study found that gives direct evidence of the interaction of bacteria and phytoplankton, Oppenheimer (44) showed by microscopic examination that clean healthy algal cells did not allow the attachment of bacteria. He examined 7000 microscopic fields from sediments from the shallow bays of Texas. The surfaces of living* diatoms, flagellates, and blue-green algae were inspected and found to be free of bacteria. However, 98% of the dead organisms had bacteria attached to them. Of several reasons for this phenomenon, Oppenheimer states that the two most likely are: 1) production of a bacteriacide, or 2) the existence of a potential on the algal surface similar to bacterial surfaces. Although evidence may be presented to support both mechanisms, Oppenheimer considered the latter to be the more likely. James (46) indicated that it was not unreasonable to assume that all living microorganisms have a negative charge similar to that of living bacteria. This was shown to be true with algae as Golueke and Oswald (47) showed that they do have a negative surface charge and could be removed from water with a cation exchange resin. This charge was also pH dependent, being greatest at 3.5 and 10.5 and lowest at pH 7.0. It was also shown that surface potential was greater with young cells than with older cells.

There is, however, ample evidence that in some cases growth inhibiting substances (to bacteria or to bacteria and algae) are produced by algae (48,49,50,51,52,53,54,55). Pratt et al. (54) first noted an extracellular bacteriacidal product from Chlorella which he called chlorellin. This same chemical was earlier implicated as a growth inhibitory substance which accumulated in old cultures (56,57). This work provided the background for a great deal of research and study on the general topic of the production of growth inhibiting substances in algae (50,51,58).

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*If cell inclusions were abnormal or missing, or if Brownian movement was observed within the cell it was considered dead (44).

In algal stabilization ponds treating sewage,the sanitary engineer takes advantage of the fact that algae survive very well in the midst of great numbers of bacteria. The algae not only survive in a healthy condition but in many cases a great reduction of total bacterial numbers has been reported (48,49). A 90% decrease in bacteria is often observed in algal ponds operating under favorable conditions, with a 60% decrease in viable cell counts in algal ponds operating under unfavorable conditions. Although it is often assumed that the bacterial reduction is due to the production of extracellular algal products, extreme fluctuations in the pH observed in an algal pond could rapidly change the number of viable bacteria also.

Although there are diverse opinions as to the exact nature of bacterial-algal interactions, most researchers agree on two points: 1) live, actively growing algae possess some unknown resistance to decomposition by bacteria, and 2) dead algae lose this resistance and are readily decomposed. Since the algal cell, in general, uses a small percentage of its cellular material for endogenous respiration, ecologists are of the opinion that in many natural systems where algal material is the primary substrate, the bacteria and microscopic animals bring about over 90% of the energy change which occurs (59).

Zooplankton-Algae Interaction

There are many species of animals that make up the zooplankton in any water body with the size ranging from the single cell protozoa to the largest fish. The fish may be dismissed from consideration because they would seldom if ever directly influence the phytoplankton population to a significant degree (60). In a consideration of the effect of the zooplankton on algal decomposition, there are two factors which must be kept in mind when considering the reported results: 1) some groups of animals are overlooked because of the difficulty of enumerating them (such as the microscopic animals), and 2) the effects of some groups such as the bacteria are often included. These two factors have the potential of masking the real significance of the organism in question. The major portion of the research on the relationship of zooplankton to algae has been completed with the relatively large algal filter feeding organisms such as the copepods and the cladocerans. A good summary of the biology of suspension feeders has been published by Jorgensen (40). There seems to be a lack of information on the effect of smaller microscopic animals such as the rotifers and the stalked ciliates--apparently because of the difficulties in studying them.

Whether zooplankton grazing (the collection of algae from water by filter feeding animals) actually controls the natural phytoplankton population has been the subject of much discussion. Some researchers believe that there is a direct relationship between cessation of algal growth and animal grazing (61). Others feel that little or no relationship exists (62,63,64,65). In any case, there is general agreement that the pulses of zooplankton observed in natural water bodies are dependent upon the pulses of the food supply--the algal mass. There have been reported two natural relationships which exist between the algal mass and the zooplankton. In one case, the algae grow, reach a maximum population, and then begin to die. The large quantity of algal mass stimulates zooplankton egg production, but because of the time lag between egg production and birth, the animals do not reach their maximum population until some time after the maximum algal population has occurred (66,67,68). The observation in nature of maximum populations of phytoplankton occurring simultaneously with minimum populations of zooplankton has led some investigators to postulate a mechanism, termed "animal exclusion," which can account for this phenomenon.

The second relationship which has been observed with phytoplankton and zooplankton is a simultaneous occurrence of the two populations. As the algal population increases the animal population increases because of the summation of reproduction and migration of the grazing animals from other regions of the water body (69). At this time there seems to be no rational explanation for the occurrence of the "animal exclusion" phenomenon or simultaneous peak populations in algae and zooplankton.

A number of contradictory observations has led to considerable confusion regarding the use of microscopic algae as a food source for suspension feeders (60,70,71). Intact and viable algal cells found in the feces of various suspension feeders has led to the suggestion that phytoplankton were only poorly utilized (60). Different species of algae have been shown to vary greatly in their food value (40), and algae that produce good animal growth in one experiment may not in the next (72). Some researchers (40,71) have indicated that the possession of thick cell walls generally reduces the food value of algae, making the cell contents unavailable to the consumers. Algae such as Chlorella and especially Stichococcus species with thick cell walls have often proved to be an inferior food when compared with naked flagellates or diatoms. To date a suspension feeder has not been found which is able to utilize species of Stichococcus as food (40). Yet these cells were found to be ingested and digested by the ciliate, Fabrea salina, which may in turn be used as food for fish larvae (73).

Another source of conflicting evidence is the method of reporting the use of the algae by various suspension feeders. When algae are eaten by animals the intake of food is not a measure of the removal of organic matter from the water for two reasons. Some of the algae are immediately returned to the water as excretory products and some of the algal matter is synthesized into new animal cells. The amount of the food that the animal uses for respiration is the only organic fraction that is actually destroyed. Both the fecal material and the newly synthesized animal tissue still remain. The consumption of algae is often reported as assimilation or as an assimilation ratio, but the reported value for this may vary, depending on the way in which it was measured. Assimilation has been generally accepted to refer to the amount of organic matter resynthesized into new animal tissue. Thus the percentage assimilation can be expressed as: [(Total food intake - Respiration - Egested material) $\div$ (Total food intake)] X 100.

A summary of some of the observed values for the per cent assimilation of algae by animals is shown in Table 5. From these results it can be seen that there is a wide range of values for various algae and for various animals.

Table 5. Assimilation of algae by various animals

ANIMAL	FOOD SOURCE	% ASSIMILATED	REFERENCE
Calanus finmarchicus	Skeletonema	40.1 to 68.1	77
Calanus finmarchicus	Syracarphaera	97	77
Calanus finmarchicus	Four species diatoms	50 to 80	77
Calanus finmarchicus	Chlorella	50, avg. 15-20	77
Calanus finmarchicus	Dinoflagellates	77-98	77
Calanus finmarchicus	Cryltomonas	53-78	78
Calanus finmarchicus	Syracosphaera	68-76	78
Artemia	Green algae-Dunaliella	Max. 53	78
Artemia	Stichococcus	0	79
Euphausia pacifica	Pacific Ocean	11.3-73.6, avg.32.5	80
Paphnia pulex	Chlamydomonas reinhardi	55.4-58.6	81
Diatomus siciloides	Natural state, fresh water	43-117 avg. 50	82
Calanus finmarchicus	Skeletonemia	53.8-67.5 avg. 61.7	83
Calanus hyperboreua	Diatoms	59.8-64.5	84
T. fluviatillis	Diatoms	68-70.2	84
Calanus hyperboreus	6 species diatoms	40.1-67.3 avg. approx. 50	84
Calanus hyperboreus	Bacteria	71.6	84
Calanus hyperboreus	Chlorophyceae	86.7	84
Palaemonetes pugio	Nitzschia closterium	78	85
Zooplankton	Natural ocean	17-90	86

Although the larger animals such as the Daphnia and the copepods may represent a large fraction of the animal mass (74) in a natural water body, the smaller protozoas and rotifers are often noted as having more significance in terms of algal degradation (75,76). In a study of the planktonic rotifers of the major waterways of the United States (as reported by the Federal Water Pollution Control Administration's National Water Quality Network), Williams (75) reported that the most numerous metazoans found were rotifers. Numbers ranged from zero (one sample) to 714 per liter with 82.1 individuals per liter on the average. In all samples studied there was a direct correlation between the number of rotifers present and the density of the phytoplankton population thus indicating that the microscopic animal population in most cases was proportional to the algal population.

The presence of animals can affect the decomposition of algae as much in an indirect manner as it does directly (59). This is seen in a consideration of the fecal material which the animal returns to the environment. As noted from the previous paragraphs, the amount of food returned as feces will vary considerably depending on the amount of organic matter respired and the amount assimilated. The fecal pellets are a highly concentrated representation of the plankton consumed and will in many cases remain intact because of a thin pellicle that is formed around each fecal pellet as it is egested. In this form nature has provided these animals with their own biological sewage treatment process. The high concentration of bacteria in the gut of the animal act on the fecal matter taking up the soluble matter and synthesizing a portion heterotrophically for other animals to ingest (85). It has been stated that the rate of incorporation of organic matter by the growth of heterotrophic and autotrophic organisms (when exposed to light) in the fecal pellets is higher than the rate of incorporation of organic matter into herbivore tissue (85). The encasing membrane of the feces dissolves slowly thus allowing the animal to escape from the end products of its metabolism. Note that although it is the physical action of the animals that cause the intimate contact between bacteria and egested organic matter, it is the bacteria that finally bring about the decomposition of the feces.

Throughout the discussion of the effects of the zooplankton, bacteria, and algae on the decomposition of algae it was seen that it is very difficult to separate the effects that a group such as the bacteria have on the overall decomposition process, especially when they are in the presence of a large animal population. Few, if any, investigators have concluded that it is the grazing of the copepods or the Daphnia that control the phytoplankton population. It is important to note that bacteria in an aquatic environment function not only as decomposers and mineralizers, but they also exist at early stages in the food chain (59). Bacteria have far reaching effects not only as an added food source but they modify food and make it palatable to the zooplankton and also produce needed vitamins (35). Thus, although one can achieve a general understanding of the potential that each group of organisms has on algal decay, the exact relationship of one group to the other is extremely unstable and difficult to predict.

Slobodkin has stated that under the most carefully designed conditions, no true equilibrium ever existed between the algal food supply and the growth of different kinds of aquatic zooplankton (87,88). Random fluctuations in animal populations were observed which could not be explained by any observable variation in the environment. Perhaps the most important factor to note with regards to the effect of various populations on algal decomposition is that the most consistent effects will be those exerted by the organisms which are generally present, i.e., the bacteria and the microscopic animals and that these two groups are believed to account for over 90% of the energy change in most natural systems (59).

NITROGEN AND PHOSPHORUS REGENERATION

The sanitary engineer designs algal ponds which are reported to extract 80 to 90% of the soluble nitrogen and phosphorus from the sewage (89,90) being treated. Nature designs her own nutrient removal system in the form of lakes and ponds. The removal of soluble nutrients in lakes and ponds has been reported to vary from 40 to 80 per cent for nitrogen and 70 to 80 per cent for phosphorus on an annual basis (90). In all of the instances where algal growth occurs and the removal of nutrients takes place, it should be noted that the nutrients which are stored for an unknown period of time in algal cells, may reappear under some conditions somewhere else. This movement of nutrients can be described as a "transfer of pollution." That is, the algal growth potential and the oxygen usage potential of the nutrients are transferred downstream to some point where the algae are subsequently decomposed and the nutrients regenerated and made available for further algal growth. This uptake and regeneration cycle is observed in the natural environment in nearly all bodies of water. Here, algae may go through extremely rapid growth periods followed by decomposition and regeneration of the algal nutrients, which may be followed once more by another period of growth. The periods of rapid growth are called algal blooms and the number of blooms for any body of water may range from none to many each year (91) depending on the state of eutrophication of the water as well as other factors.

Nutrient regeneration is a concomitant by-product of the carbon regeneration processes which takes place during algal decomposition. For this reason nutrient regeneration should not be considered separately from carbon regeneration. However, few studies have been extensive enough to define the change of carbon as nutrient regeneration takes place.

Quantity of Nitrogen and Phosphorus Regenerated

Van Brand and others (92, 93, 94, 95, 96, 97, 98, 99, 20) have either collected natural algae or have grown algae in natural waters (with the natural bacteria present) in the laboratory and then have placed them in the dark and monitored the resulting changes in either nitrogen and/or phosphorus for periods varying from several weeks to 414 days. The resulting changes in the particulate organic and soluble nitrogen and phosphorus during the decomposition of the algal mass are summarized in Figure 5.

Three general zones of action may be seen to occur. When the algal organic mass is initially placed in the dark, a small quantity of nitrogen and phosphorus may be released to solution within the first 24 hours (less than 5% of the total particulate phosphorus was reported to be released in 24 hours by diatoms in one study (99)). This release ceases quickly and a transition of soluble phosphorus and nitrogen to particulate organic nitrogen and phosphorus then takes place. This absorption process increased the organic nitrogen and phosphorus by 11 and 34%, respectively, over the initial value in approximately eleven days in Grill and Richards study (99). A further complication in regeneration of

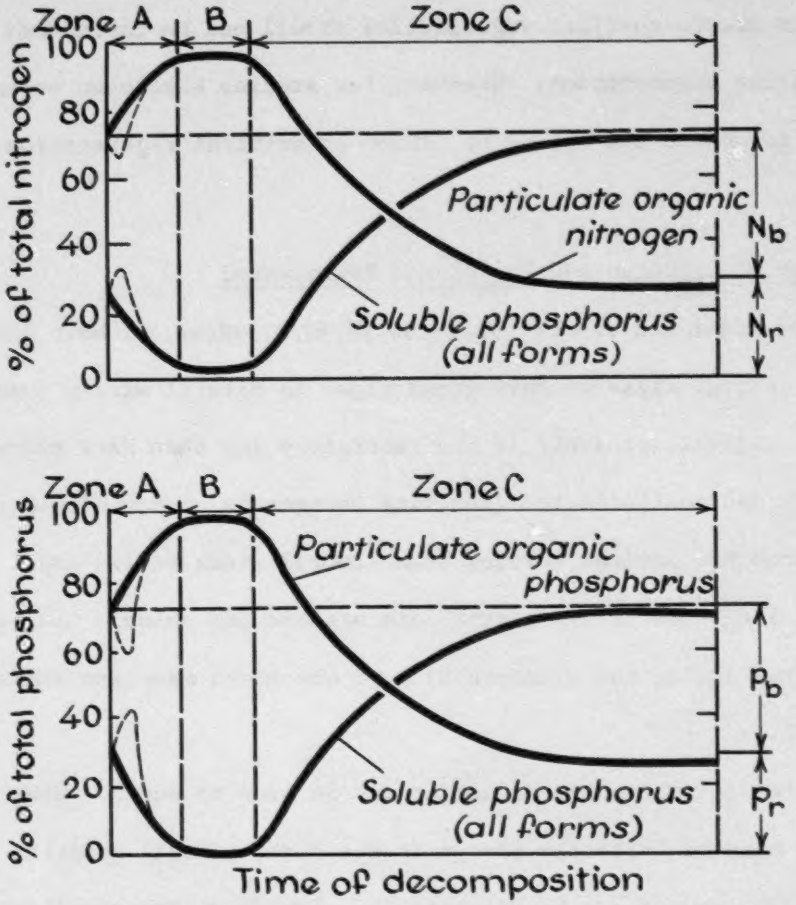


FIGURE 5. Typical nitrogen and phosphorus changes during the decomposition of phytoplankton in the dark. N_b and P_b represent the quantity of nitrogen and phosphorus regenerated, and N_r and P_r represent the quantity of nitrogen and phosphorus not regenerated.

nitrogen and phosphorus occurs when it is noted that during some of the early growth stages algae may continue to rapidly absorb nitrogen and phosphorus from solution for several days when placed in the dark (100,102 103,104). The zone where these relatively rapid exchanges took place is designated as zone A in Figure 5.

Following zone A, a stationary phase is sometimes observed to occur where apparent nutrient regeneration is zero. This is designated as zone B in Figure 5. Finally, zone C is shown as the zone where active regeneration is taking place. When the nutrient regeneration processes cease, indicating that biological activity is approaching zero, some of the initial nutrients remain in the particulate organic material. The remaining particulate organic nitrogen and phosphorus is designated as N_r and P_r, respectively, in Figure 5. Note that Figure 5 combines all features of nutrient regeneration that have been observed by other researchers, but that in any given instance all three activity zones may not have been observed.

One of the most significant results of all the studies on nitrogen and phosphorus regeneration is the finding that a significant fraction of the initial algal particulate nitrogen and phosphorus is not regenerated. Van Brand and others (20,93,94,95,96,97,99) report values of 16 to 35% of the initial particulate nitrogen remaining after decomposition (N_r) for a wide variety of phytoplankton in both ocean water and fresh water. The decomposition resistant phosphorus (P_r) has been reported in four cases to be approximately 33 per cent (20,92,98,99), or in other words, two thirds of the initial algal organic phosphorus was regenerated. Although some investigators have briefly speculated about the reason for the occurrence of N_r and P_r, no complete explanation is available. Grill and Richards (99) in one of the most comprehensive treatments of nutrient regeneration assumed, in a mathematical model which they developed (to be discussed further, subsequently) that N_r and P_r originate from bacterial activity and that the quantity of N_r and P_r remain constant throughout the regeneration process.

Rate of Nitrogen and Phosphorus Regeneration

Less information is available regarding the rate of nitrogen and phosphorus regeneration from phytoplankton. Using a first order decay rate coefficient and the reported half lives (time period to regenerate half the available nitrogen and phosphorus, N_b, P_b) it can be seen that both nitrogen and phosphorus regeneration have been observed to occur at a rate varying between 0.038 and 0.17 day^{-1} (20,93,99). Based on the available information it would appear that there is not a good explanation for nitrogen and/or phosphorus regeneration and the only way presently available to approximate the regeneration rate is through empirical measurements.

Grill and Richards (99) have developed an empirical model which reproduced their laboratory nitrogen and phosphorus regeneration experiments carried out with naturally decaying diatoms in sea water. Phosphorus regeneration was assumed (on the basis of the shape of the curve of the results) to be the resultant of the decay, at first order rates, of two labile organic fractions, P_1 and P_2, ($P_1 + P_2 = P_b$, in Figure 5). It was also assumed that a refractory fraction, P , was present and remained constant. The assumed mechanism of release for phosphorus was as follows:

$$\begin{tikzcd}
 & & & P_i \\
P_I \arrow[r, "K_1"] & P_x \arrow[r] & P_B \arrow[ur, "K_2"] \arrow[dr, "K_3"'] & \\
 & & & P_r
\end{tikzcd}$$

where P_I was the available phosphorus in the diatom tissue (P_b in Figure 5). P_I decays at a first order rate, K_1. The phosphorus released into solution by the diatoms before accumulation by the bacterial organic material P_B, was represented by P_x. However, the rate of bacterial assimilation appeared to be so rapid that the concentration of P_x was always negligible. Bacterial respiration and the decay of dead bacterial cells gave rise to the inorganic phosphate fraction (P_i), and the refractory organic substances (P_r) which was synthesized during bacterial metabolism and which was assumed to be the remains of dead bacterial cells. The sum ($K_2 + K_3$) described the rate of loss of phosphorus from the bacterial fraction to the two product fractions formed in constant relative proportions. The governing differential equations for this model were:

$$\frac{dP_I}{dt} = -K_1 P_I$$

$$\frac{dP_B}{dt} = K_1 P_I \quad -(K_2 + K_3)\, P_B$$

$$\frac{d}{dt}\,(P_i + P_r) = (K_2 + K_3)\, P_B$$

Using trial and error for determination of the rate coefficients and other curve fitting techniques, Grill and Richards were able to show that first order kinetics would fit the observed data. This approach to defining nutrient regeneration is of little use in other circumstances since no basic explanation of the regeneration mechanism was given. The important contribution of this work is to note that phosphorus regeneration through bacterial action may be described by first order kinetics.

FACTORS CONTROLLING NITROGEN AND PHOSPHORUS REGENERATION

The mechanisms controlling nitrogen and phosphorus regeneration have been discussed by several researchers with some common agreement. As early as 1934 Redfield (105) demonstrated that the changes in phosphate and nitrate in the ocean occurred in fixed relative proportions. Because of these results Redfield postulated that during growth and decay of phytoplankton, nitrogen and phosphorus are removed and released in a fixed ratio of 16:1 (on an atom basis). Thus this hypothesis would indicate that the rate of nitrogen regeneration should be 16 times the phosphorus regeneration rate on an atom basis. Subsequent work showed that this was often the case as the ratio of the rates of nitrogen and phosphorus regeneration from decomposing diatoms in Grill and Richards' study (99) had an atom ratio of 16:1, over most of the study; but significant variations were observed at the beginning and end of decomposition. Riley (106) and others (107) have shown that this ratio can vary over a wide range in the Atlantic Ocean and that it is far from being a constant number as postulated by Redfield. A rational explanation for the occurrence or non-occurrence of such a ratio seems to be based on the uniform composition of most algal genera, but this explanation sheds little light on the mechanism of nutrient regeneration.

Bacterial and Zooplankton Effects on Nitrogen and Phosphorus Regeneration

Von Brand and others (20,93,99) have stated that bacteria exert a great influence on the regeneration process. Skopintzer and Broock (20) in a laboratory study showed that the bacterial population seldom exceeded the mass which occurred in natural water during algal decomposition and that the greatest number of viable bacteria occurred in the algal culture after 2 to 5 days in the dark. Von Brand _et al_. (93), Skopintzer and Broock (20), and Grill and Richards (99) all agreed that in zone A shown in Figure 5, the initial release of nutrients is an autolytic mechanism, and that the subsequent conversion to particulate organic nitrogen and phosphorus is due to bacterial growth. Grill and Richards (99) attributed zone B to the incorporation by bacterial growth of all the nitrogen and phosphorus which is autolytically released by the dying phytoplankton population, resulting in little if any regeneration. The length of zone B and the cause for its cessation remain unexplained.

Whether nitrogen and phosphorus are regenerated from algal material as a result of algal autolysis, algal enzymatic action, bacterial enzymatic action, or through the grazing of animals has been considered in some studies but no clear answer is available. Corner _et al_. (80) in a detailed study of the Copepod, _Calanus finmarchicus_, found that during the life span (10 weeks) of one of these animals less than a quarter of the nitrogen removed from the water as algal cells was resynthesized into animal tissue and the production of eggs. The rest was lost as fecal pellets, moults, and waste products of metabolism. It might be concluded from this study that the major effect of larger animals on nitrogen regeneration would be to prepare the algal cells for further and more rapid decomposition by bacteria and microscopic animals.

Barlow and Bishop (108) have stated that the regeneration of phosphorus by cladocerans in the epilimnion of a fresh water lake was sufficient to support the requirements of the phytoplankton population during that period of measurement. However, the authors did not indicate the relative effect of bacteria on microscopic animals during the period of measurement. Thus their conclusion is subject to question. Pomeroy _et al_. (109) attempted to control the bacteria population with penicillin (but did not eliminate it) in another study of the effect of zooplankton on phosphorus regeneration in a fresh water lake. These researchers also concluded that phosphorus regeneration by zooplankton was great enough to satisfy that required by photosynthesis.

Hayes and Phillips (110) have indicated the complexity of the zooplankton-algae interaction by reporting that zooplankton cannot use inorganic phosphorus until bacteria have made it organic. Others have shown that animals fed algae in the absence of bacteria have little uptake of nutrients whereas with bacteria present there is a much higher uptake thus implicating the importance of bacteria living in the guts of some animals (111).

Johannes (112) in a study of the effect of marine protozoa on phosphorus regeneration from algae has noted the delicate balance that occurs between algae, bacteria and ciliates. This author reported that a greatly increased rate of phosphorus regeneration occurred when ciliates and bacteria were both present than when only bacteria were present. The explanation which Johannes gave for this increase was that the grazing protozoa kept the bacterial population in a "physiological youth" state and that in this state the rate of phosphorus regeneration was higher than when bacteria grew slower over longer periods of time. It was noted in this experiment that the actual release of phosphorus by the bacteria was less than 1 per cent, whereas 31 to 85 per cent of the total particulate phosphorus was released as dissolved inorganic phosphorus in 9 to 10 days in the bacteria-ciliate system. In another experiment Johannes (113) reported that with marine bacteria grown over a period of 15 days, the population declined 90% but only 5% of the phosphorus originally held in the cells was regenerated. Thus Johannes has concluded that either living or dead marine bacteria release little dissolved organic phosphorus, but that ciliates increase regeneration by ingestion of bacteria and subsequent excretion of dissolved phosphorus. The failure to explain the mechanism of phosphorus regeneration based on such fundamental factors as the quantity and state of the energy source does not allow generalizations to be made about the relative effect of bacteria and ciliates on phosphorus regeneration. In another study this author has shown that the smaller microzooplankton are much more effective in regenerating phosphorus than are the larger zooplankton (114).

Phillips (115) in a study of the role of microorganisms in the phosphorus cycle in water supported the hypothesis that bacteria control the reversion of organic phosphorus by a series of reversible first order reactions as shown below:

$$\text{Inorganic \& Part.P} \rightleftharpoons \text{Bacterial organic P} \rightleftharpoons \text{Soluble Organic Phosphorus}$$

The author noted that estimates of bacterial numbers always indicated that the dissolved organic phosphorus maximum occurred during a decline in bacterial numbers. These results were shown to hold for a system with marine bacteria and a pure culture of algae (Nostoc), when bacterial growth was stimulated by adding nutrient broth to sea water (115). No dissolved organic phosphorus appeared during the growth phase, suggesting that living bacteria and phytoplankton may not release organic phosphorus. The appearance of significant quantities of dissolved organic phosphorus was observed with a decline in cell numbers and was presumably due to the death and decay of microorganisms. It was further shown that the bacteria were the main agents in converting the dissolved organic phosphorus into dissolved inorganic phosphorus. The author noted that over a period of 7 days, 20% of the dissolved organic phosphorus was converted to dissolved inorganic phosphorus. These results clearly indicate the significance of the presence of a usable substrate and the mass of the decomposing organisms as controlling variables with phosphorus regeneration. It was concluded that outside influences such as bacterial enzymatic action and the death and decay of the decomposers were responsible for most regeneration (115).

Shapiro (116) has noted that bacteria and mixed microorganisms actively release a large proportion of their phosphorus to the medium in a matter of hours under anoxic conditions. Only phosphorus was lost, probably as orthophosphate and apparently mostly from the acid-soluble fraction of the cells. This process was easily reversible when the bacteria were returned to aerobic conditions. This research emphasizes the delicate state of the phosphorus in bacterial cells and the fact that the condition of an animal's digestive tract is perhaps harsh enough to cause bacterial release of phosphorus. However upon release to the water, bacteria will also probably reabsorb the regenerated phosphorus, depending on the state of the substrate used for energy. The main fact which is not discussed in most of these studies is the quantity and state of the bacterial energy source. If an energy source is available bacterial release of phosphorus occurs at a very low rate. If, however, the energy source becomes scarce while animals are preying on the bacteria, the phosphorus may be rapidly released to solution.

Another factor which has been shown to be significant in relation to phosphorus regeneration is the concentration of phosphorus in the algal cell (98). With algal cells containing a minimum amount of phosphorus, little phosphorus regeneration was observed during decomposition. Thus the age and the nutrient concentration in the algae are also factors which must be considered in a study on nitrogen and phosphorus regeneration.

EFFECT OF TEMPERATURE ON ALGAL DECOMPOSITION AND NUTRIENT REGENERATION

In general, an increase in temperature results in an increase in the rates of any given biological system over a narrow temperature range. In 1889, Arrhenius examined the available data on the effect of temperature on the rates of chemical reactions and proposed the following equation:

$$\log_e K_2/K_1 = A\,(T_2-T_1)/(RT_1T_2)$$

where K is the reaction velocity constant, R is the gas constant (1.987 calories per degree per mole), T is the absolute temperature (Kelvin degrees), and A is a constant.

Because of the complexities of the numerous chemical reactions occurring in any biological system, the value of "A" may not have much theoretical significance. Several simplifying modifications of the above relationship have been utilized. Within the range of natural temperatures encountered in sewage and water, Streeter and Phelps (117) showed that the factor $A/R(T_1T_2)$ is essentially constant. Denoting this constant as B and changing logarithmic bases, the above equation may be written:

$$\log_{10} K_2/K_1 = B(T_2-T_1) \text{ and } K_2/K_1 = 10^{B(T_2-T_1)}$$

Replacing 10^B by θ yields the following equation:

$$K_2 = K_1 \, \theta^{(T_2-T_1)}$$

For convenience of comparison, one more simplification is often used. When $T_2-T_1 = 10$, let $Q_{10} = \theta^{10}$. This final relationship becomes: $K_2/K_1 = Q_{10}$. A frequently used "rule of thumb," enumerated by van't Hoff is that Q_{10} for many reactions equals 2. That is, the rate of a reaction doubles for each 10°C rise in temperature.

Few studies have included as part of their objective the definition of the effect of temperature on algal decay or nutrient regeneration. The Q_{10} for several biological systems is noted in Table 6. As shown in this table the rule of thumb of a doubling of the rate of reaction for these biological systems is a good approximation of the data. The maximum range of temperatures of interest with natural algal systems would be from 0° to approximately 55°C with most natural bodies ranging in temperature from 0° to 30°C. The highest temperature for optimum growth of one species of algae was reported to be 41°C (113).

Table 6. Effect of temperature on the oxygen uptake rates of various organisms and biological systems

BIOLOGICAL SYSTEM	Q_{10}	REFERENCE
River Ecosystem - Photosynthesis	2.3	121
Respiration	0.0	
Green Algae - Respiration	1.7-2.4	122
Periphyton algal community - temp. increasing	2.5	123
temp. decreasing	1.84	
Decomposition of organic nitrogen compounds	2.5	95
Reversible release of phosphorus from bacteria and other mixtures of microorganisms	2.0-2.5	116
Diatomus siciloides Lilljeborg - respiration	2.95	82
Aquatic plants - respiration	1.5-2.3	
Natural Algal decomposed by Bacteria and Animals	2.43	20

AGE AS A CONTROLLING PARAMETER IN ALGAL DECOMPOSITION AND NUTRIENT REGENERATION

Some of the research reported on the aging of algal cultures indicates that this variable may be one of the major environmental controls in their decomposition. There are, in general, two ages involved with algae: 1) culture age, and 2) cell age. Unless the algal culture is completely synchronous these two ages are very different. Age has meaning in reference to a yeast cell since it has been shown that the number of bud scars on a yeast cell controls the number of times that the cell can divide (119). When the cell has many scars it can be called an old cell. At the present, however, age has no meaning, in this context for algal cells (120). It is culture age or community age that is considered the important parameter and this is usually reported as a density variable.

Age here will always refer to the amount of time that an algal culture was allowed to grow or decay under a given set of environmental conditions. In this context it should be noted that culture age will be influenced by nutrient depletion as much as by any other variable. Since the effects of time and nutrient depletion are inseparable in the natural environment it would be of little benefit to try to separate these two effects. Instead it has been assumed in most studies that nutrient depletion and cessation of cell division is synonymous with age.

The most obvious change with respect to age in algal cultures is in the composition. In some cells accumulation of lipid increases from 10% in young cells to more than 80% of the dry weight in older cells (125, 126). During the stationary phase many algae excrete a significant fraction of their cell carbon into the environment. Lewin (127) in a study of the production of extracellular polysaccharide found that 20 to 50% of the total oragnic matter was given off by young green algae. This author also separated the mucilage around the cells of some species and reported that the mucilage amounted to 40 to 60% of the total organic matter in older cultures. In laboratory studies she observed that as these cultures aged the mucilage content of the cultures became so high as to affect the viscosity of the medium. Grillard and Wangerdsy (128) also reported that naked marine algal flagellates in the senescent stage (old, none growing state) produced a significant amount of extracellular material.

Photosynthesis and respiration rates both decrease rapidly as algal cultures age in the light with little concomitant decrease in cell mass (125,129). Pratt (56,57) noted that culture photosynthesis varied from a maximum of 100% in young cells of 0 to 4 days to a constant of 35% of its initial value in 20 days. Respiration also varied from 100% to a constant value of about 40% over the same time period. A further note on the effect of age on young growing cultures was demonstrated by Pratt (57,61) when he introduced old cells into an active young culture and he observed a significant inhibition of growth. Fogg (129) indicated that age changed the respiration rate of Monodus subterraneous from an average oxygen uptake rate of 6.5 ul O_2/mg/hr to less than 0.2 in 40 days. There was also a significant change in photosynthesis and respiration as each individual cell aged during its growth phase (129,130). The lowest rates were observed in small daughter cells (light cells) at the start of the life cycle. As these cells grew, photosynthesis reached a peak in 3 to 4 hours and then there was a decline to the lowest value which occurred at the time of cell division (dark cells). There is little practical importance in this phenomenon except to note this source of variability in the testing procedures.

Perhaps the most meaningful change with age is reflected in the effect of senescent algal cells on the feeding rates of some of the animals in water (77, 84,131,132,133). Ryther (131) showed that a combination of senescent cells at high concentration could change the feeding rate of Daphnia from 81 ml per animal per day to a value approaching or equaling zero. All feeding of Daphnia exposed to senescent Chlorella ceased in 12 hours in all experiments. All animals died after 11-13 days when grown on senescent algae. Similar results were obtained in studies on other animals (77,84,131).

From these results it could be inferred that the age of an algal culture has a major control over its biodegradability. However, little more can be concluded from existing studies than that biodegradability decreases with age of algal cultures. To date, studies have not been conducted to determine the effect of the algal age on nutrient regeneration.

SUMMARY

The production of excess phytoplankton in many of the water bodies of the United States has caused numerous significant problems with few answers being provided by previous research. The engineer has little idea what quantity of algae can be produced in any given water body. Also lacking is a definition of algal decomposition, which is important in handling the problems associated with oxygen uptake, and nitrogen and phosphorus release with which the engineer is faced.

Once algal growth has occurred it has been shown that between one and two thirds of the algal organic matter resists decomposition. In other words, the algal mass used between one third and two thirds of the quantity of oxygen given off during photosynthesis in stabilization of the algal organic mass. It can be seen that this becomes a significant factor in the determination of the oxygen balance of a water body. No studies have explained on a rational basis the reason for the formation of the organic material which is resistant to biological decomposition nor its significance in the overall decomposition process.

The rate of decomposition of natural algae has been the subject of several short studies. In all cases the specific decomposition rate coefficient (first order decay rate coefficient, $K_{\text{base e}}$) varied between .01 and .08 per day. Although none of these studies discuss the rate controlling steps in the algal decomposition process, the information indicates that algae are always characterized by a slow controlled oxidation process.

The rate and quantity of nitrogen and phosphorus regeneration from phytoplankton are two very important factors which influence the complex nitrogen and phosphorus cycles in water. Of more practical interest to the sanitary engineer is how long it takes the nutrients that cause initial algal growth to become available again, and what quantity becomes available. The current literature indicates that of the total nutrients initially in the algal cells, 65 to 84% of the nitrogen and about 67% of the phosphorus can be regenerated. The rate at which regeneration takes place has been reported to range from 0.03 to 0.17 per day (first order rate coefficient, base e). No studies have clearly indicated the controlling mechanisms of nutrient regeneration.

Thus two factors become clear in a consideration of the effect of algal decomposition on the carbon, oxygen, nitrogen and phosphorus resources of natural water. First, it has been demonstrated that algal decay is a serious problem and in an uncontrolled and undefined state can lead to serious consequences such as deoxygenation of waters, perhaps resulting in large fish kills. (212). Secondly, the information which is presently available emphasizes that algal decomposition and accompanying nitrogen and phosphorus regeneration is still vaguely defined. The reason for the resistance to biological decomposition of the large fraction of algae also remains almost completely unknown.

III. THEORETICAL CONSIDERATIONS

A theoretical consideration of the effect of algal decomposition on the oxygen, carbon, nitrogen and phosphorus resources of a water body can be divided into four distinct areas: 1) Algal growth kinetics, 2) Algal yield (mass produced), 3) Algal decomposition kinetics, and 4) Nutrient regeneration resulting from algal decay. Although the decomposition of algal systems is the main subject of this study, information from algal growth kinetics is necessary to properly define the characteristics of the organic material produced by the algae, and information regarding the algal yield or the amount of algal organic material produced will indicate the intensity of the effect of algal decomposition. Algal growth kinetics will only be briefly considered with the other three areas: algal yield, algal decomposition, and nutrient regeneration, which are discussed more fully on a theoretical basis. An explanation of the factors controlling the processes involved in each area will be presented along with equations which can be used for predictive purposes.

ALGAL GROWTH AND DECOMPOSITION

Algal Yield

Although the extent of algal growth in natural waters from a given quantity of nutrients is of interest, no adequate explanation or method of estimating yield of algae is presently available. A theoretical approach to the definition of algal yield and the manner in which the empirical data provided in this study may be used to predict this yield are summarized in the following paragraphs.

Many studies of algal nutrient relationships have been made. Ketchum attempted to precisely define four nutrient relationships as follows (139):

1. "The absolute requirement for a nutrient is based upon the postulate that the algae cannot grow, reproduce, or photosynthesize if the nutrient is lacking from the environment, and that no other nutrient can be substituted for it.
2. The normal requirement is the quantity of each nutrient contained in cells produced during active growth of a population while no nutrient is limiting.
3. The minimum requirement is the quantity of a nutrient in the cell when the nutrient is limiting the growth of the population, all other nutrients being present in excess.
4. The optimum concentration or range of concentrations will permit the maximum rate of growth, reproduction, or photosynthesis of an algal population."

The "absolute requirement" means the nutrient must be present before the algae can grow. The "optimum requirement" refers to the concentration of a nutrient in the media surrounding the algal cell; and the "normal requirement" and the "minimum requirement" refer to the concentration of nutrients in the cellular material.

Before considering the significance of the various algal nutrient requirements, it is important to note several other factors which affect algal yield in natural waters on a given quantity of nutrients. Other populations will affect the natural algal yield in two ways: 1) by competing for the available nutrients, and 2) by predation. Whether the bacteria and animals successfully compete for the available nutrients and thereby decrease algal yield will vary depending on the source of energy available to the bacteria and animals. If organic pollutants, other than algae, are present, bacteria and animals may form all or a major fraction of the living organic mass present. However, it has been shown that the bacteria and animal mass seldom comprise more than one per cent of the total organic material present in an algal system lacking in energy sources other than light. In this case it can be assumed that the available nutrients used by the decomposers are small in regards to the total available.

Physical parameters have the greatest potential for controlling the maximum yield from algal growth. Temperature, illumination, and circulation all can cause cessation of algal growth. All of these parameters vary in space and with time. In any situation, the engineer must consider not only the quantity of algae that does occur but also the maximum amount that might occur if all conditions were favorable. The amount of algae produced under a given set of natural conditions may be estimated by "in situ" studies. However, it is not necessary to consider the vast number of physical variations when one is attempting to predict the maximum boundary limits, in this case, the maximum algal yield.

If it is assumed that both the physical parameters and the bacteria and animals do not significantly affect the maximum algal yield, then this leaves the quantity of nutrients as the major controlling factor. According to Liebig's Law of the minimum (135) any required nutrient has the ability to limit algal growth. Lund (136) has reported evidence which indicates that the dissolved silica concentration may control the yield of Asterionella Formosa hass. The absence of the exotic elements such as molybdenum may eliminate the growth of some genera or species; whereas the absence of sulfur may limit the growth of another genera or species. Two factors cancel the limiting effect of most of the elements required for algal growth. First, if one genus or species has its growth limited by some trace element, another is probably always present to replace the growth inhibited population. Acclimation of the algae to the nutrient deficient atmosphere also may allow the same algae to continue to grow. Secondly, most natural waters probably contain enough of most of the necessary elements to support the growth produced by the critical element or elements--i.e., the elements required in greatest proportion in relation to the amount present in the system (1, 137). Most researchers agree that nitrogen and phosphorus are the two most critical elements under most natural conditions (1,138,139,140). The data are divided as to whether both elements are equally important in promoting algal growth or whether one is more important (139,141,142). Thus it can be seen that maximum algal yield in natural waters can, in general, be discussed in terms of the available nitrogen and phosphorus assuming all other conditions are favorable to growth.

A number of investigators have attempted to define the concentration of nitrogen and phosphorus in the medium which will just allow algae to grow, i.e., the minimum concentration necessary to satisfy the "absolute requirement" (134). One of the earliest and best studies on the influence of the mineral composition of the medium on the growth of planktonic algae was carried out by Chu (143,144). In these studies Chu simulated natural waters using pure inorganic salts and measured the resulting growths. He determined the concentrations of nitrogen and phosphorus to satisfy the "absolute requirement" to produce the "optimum concentration" and also the high concentration at which inhibition of growth resulted. The limiting and inhibiting requirements of nitrogen and phosphorus found were nearly the same for many different planktonic algal species, with only minor differences in the lower and upper limits. Fourteen species of fresh water algae flourished for the length of the experiment (over 2 years) in media with a nitrogen content of 1 to 7 mg/l, and phosphorus content of 0.1 to 2 mg/l. It was found that the species studied were all likely to suffer a deficiency when the concentration of nitrogen was below 0.2 mg/l N and that of phosphorus below 0.05 mg/l P. A growth inhibiting effect was observed when the concentrations of nitrogen (as nitrate) and phosphorus exceeded 20 mg/l.

Sawyer (140) and others (98,100,143,149) have reported that the minimum concentration of nitrogen and phosphorus in solution which will permit algal growth is approximately 0.2 and 0.01 mg/l, respectively. Thus when the nitrogen and phosphorus reach and exceed these minimum concentrations, algal growth can be expected to occur, other conditions being favorable to growth.

Figure 6 represents a typical growth curve for algae from a given quantity of nutrients and illustrates the changing fraction of the nitrogen in the algal cell. This illustration is based on the assumptions that no other variable such as light, or nutrients other than nitrogen is limiting. A similar curve could be drawn assuming phosphorus was the limiting nutrient.

During the early stages of growth the concentration of nutrients in the cell may be equal to or greater than the "normal requirements" (N_n in Figure 6 refers to the normal nitrogen requirement). A review of numerous articles (see Table 7) indicates that for many kinds of algae the normally required concentrations of nitrogen and phosphorus in the algal material

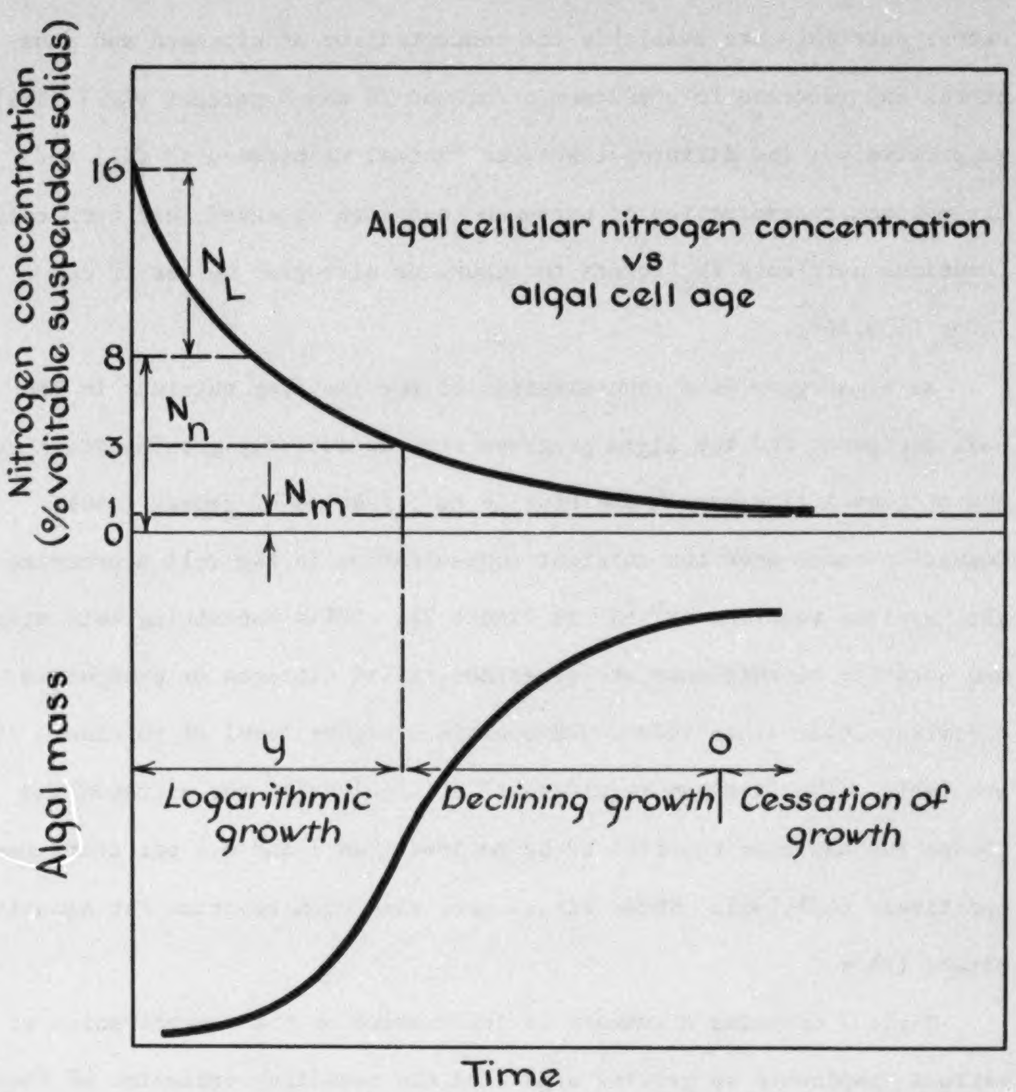


FIGURE 6. The nitrogen composition of algal cells during growth on a given quantity of nutrients. N_L is luxurious nitrogen, N_n is normally required nitrogen, N_m is minimum required for nitrogen. "y" and "O" refer to young and old cells as designated in the nutrient regeneration hypothesis.

are approximately 8 and 2 per cent (VSS), respectively. However, when excess nutrients are available the concentration of nitrogen and phosphorus may increase to a maximum of around 16 and 5 percent (VSS) (148), respectively. The difference between "normal requirements" (N_n) and the maximum concentration of nitrogen sometimes observed, has been called luxurious nutrients (N_L refers to luxurious nitrogen) by Gerlof and Skoog (138,169).

As algae grow, the concentration of the limiting nutrient in the cell decreases and the algae progress from an actively growing stage to one of restrictive growth and finally to cessation of growth (146). Cessation comes when the nutrient concentration in the cell approaches the "minimum requirement" (N_m in Figure 3). Cells containing this minimum quantity of nutrients are sometimes called nitrogen or phosphorus deficient cells since they would contain a higher level of nutrients if available. The "minimum requirement" of algal cells for nitrogen and phosphorus has been reported to be no less than 1 and 0.1 per cent, respectively (138,148). These limits have also been reported for aquatic plants (149).

Table 7 contains a summary of information on the concentration of various components in growing algae and the resulting variation of these elements under various extreme conditions. Note that the concentration given for actively growing algae may be taken as the "normal requirement" in most cases and that the lowest values may be taken as an estimate of the "minimum requirements."

Table 7. Average composition of actively growing algae and maximum variations in composition observed (per cent ash free dry weight).

SUBSTANCE	ACTIVELY GROWING CELLS	MINIMUM	MAXIMUM
C	53	42.9	70.2
H	8	6.0	10.5
O	31	17.8	34.0
N	8	0.6	16.0
P	2	0.16	5.0
Ash	10	1.5	45.0
Protein	55	3.7	88.2
Lipids	10	1.8	85.6
Carbohydrate	35.0	4.1	38.5

The above values were determined from a review of the following references: 100,105,125,126,129,134,138,147,148,150,151,152,153,154,155,171.

The "minimum requirement" of a given nutrient has practical significance since if it were known, the maximum yield of algae under conditions favorable for growth could be estimated and therefore the maximum effect of algae on the environment could be predicted. There are two factors which indicate that it should be possible to define this level of growth. First, all algae are quite similar in composition and in their biochemical mechanisms (147,30). Thus the concentration of nutrients corresponding to the level of growth as previously defined might apply to all algal genera, with a minimum degree of variation. Secondly, the difference in the activity (or turnover rate) of nitrogen and phosphorus in biological material indicates that there could be a clear distinction between nitrogen and phosphorus growth dependency. Because phosphorus is so active in biochemical material (turnover time of less than a minute in bacterial and algal cells (110)), it is proposed that nitrogen is often of more significance than phosphorus in limiting the yield of algal systems. Chu (143,144) and others (134,142) have emphasized that this might be the case by noting that phosphorus only appears to limit the yield and the rate of growth at concentrations in solution that approach the "absolute minimum requirement" of 0.01 mg/l P. Assuming that nitrogen were the controlling nutrient, then the maximum algal yield would be directly proportional to the available nitrogen and a relationship could be written: $C = a(N)$ Eq (2)

where C refers to the maximum yield of algal growth (measured in this study as mg/l of chemical oxygen demand, COD), "a" is an empirically defined coefficient corresponding to the COD to nitrogen ratio of cells when the nitrogen in the algal mass reaches the "minimum" concentration, and N is the available nitrogen (total of ammonia, nitrite, and nitrate nitrogen plus the available organic nitrogen in the water). This expression assumes the presence of at least a minimum amount of phosphorus in the algal cell material, the exact level of which must be empirically determined.

Algal Decomposition

As noted earlier a complete description of the algal decomposition kinetics must include the total quantity of material which takes part in the reaction and the rate at which the reaction takes place. These two factors are discussed in the following paragraphs.

As shown in Equation 1 if one hundred per cent of the algal material formed by photosynthetic fixation of carbon is decomposed aerobically, the same amount of oxygen would be required as was produced during photosynthesis:

$$aCO_2 + cNO_3^- + ePO_4^{-3} + (c+3e)H^+ + 1/2(b-c-3e)H_2O \underset{\text{AEROBIC DECOMPOSITION}}{\overset{\text{GROWTH}}{\rightleftarrows}} C_aH_bN_cO_dP_e + (a + b/4 + 5c/4-d/2 + 5e/4)O_2 \quad \text{Eq (1)}$$

During this process all the nutrients held in the organic material would be regenerated. However, there is abundant evidence which indicates that complete biological oxidation of cellular material rarely occurs. For example, the vast petroleum deposits on Earth have been directly attributed to the accumulation of unoxidized algal material (156), and coal deposits of 10^{12} tons are believed to be derived from swamps which covered the land eons ago (157). The fact that a large pool of undecomposed organic matter has arisen from the growth of algae suggests that perhaps the rate of oxidation of a significant fraction of plant material is so slow that for practical considerations it may be considered to be zero.

The fraction of organic material which offers an unusually high resistance to biological decomposition (i.e., when the amount of material oxidized over a time span of about a year is negligible) will be referred to as the refractory organic material. Other terms which have sometimes been used are: recalcitrant molecules, inert organic material, inert residue, and nonbiodegradable organic material.

Figure 7 defines schematically the different fractions of biodegradable and refractory material and their changes as algae are decomposed. When algal material is first produced by photosynthesis it may be assumed that the total organic material is composed of two fractions: one which is susceptible to biological decomposition (biodegradable fraction = B), and the other which is resistant to biological decomposition (refractory organic fraction = R_a). The biodegradable fraction may serve two purposes with heterogeneous communities in natural waters. One portion of the biodegradable algal cell material may be used for energy for maintenance of the algal cell by endogenous respiration. The

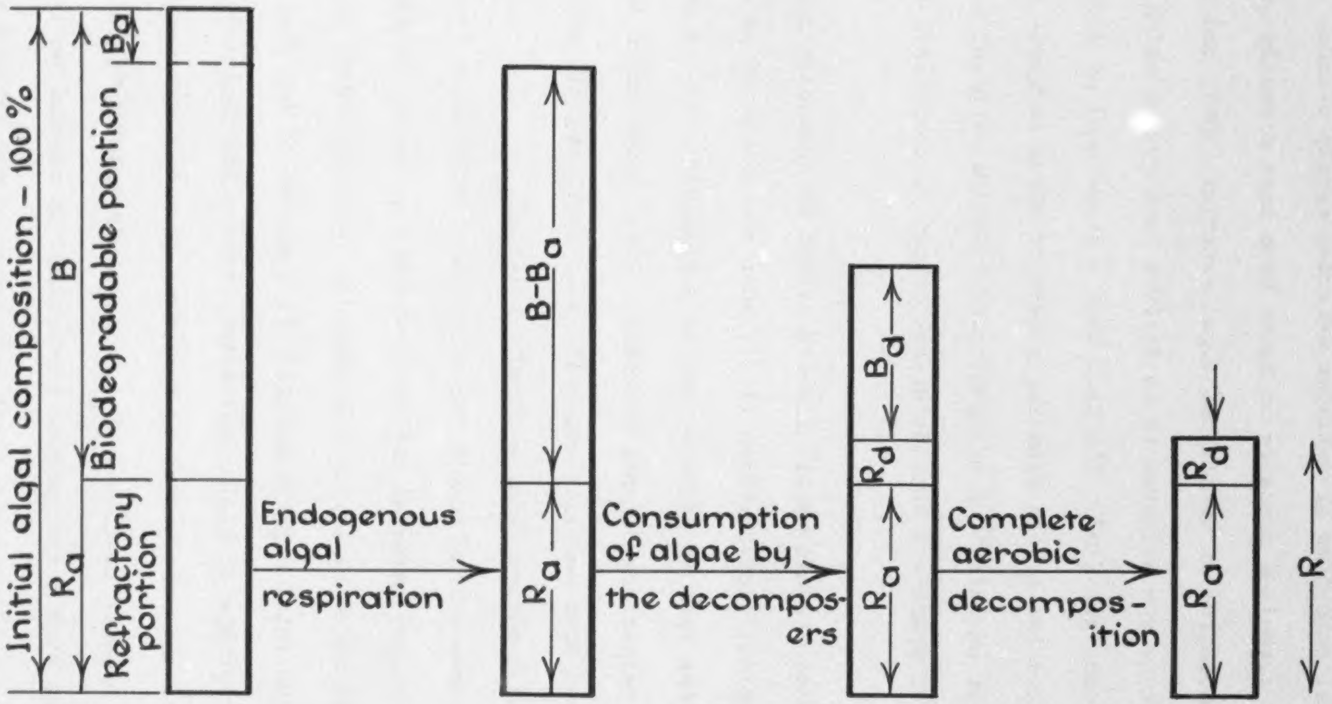

Initial algal composition – 100 %
B
Biodegradable portion
B_a
R_a
Refractory portion
Endogenous algal respiration
$B-B_a$
R_a
Consumption of algae by the decomposers
B_d
R_d
R_a
Complete aerobic decomposition
R_d
R_a
R

FIGURE 7. Illustration of the composition of algal systems as related to the biodegradability of algae at different stages of decomposition. B is the total biodegradable fraction of algae, B_a is the biogradable material used by the algae, and B_d is the fraction of B resynthesized by the decomposers. R_a is the refractory algal material and R_d is the refractory decomposer material.

other portion may be used by the decomposers (which are composed mainly of bacteria and microscopic animals) for energy and synthesis. During the synthesis of the decomposers (which is defined as secondary productivity by Odum (36), another refractory organic fraction will be formed. (R_d). Thus after complete decomposition of algae the remaining organic material (R) will be composed of refractory organic material originating from the algal cells (R_a) and from the decomposers (R_d).

The refractory organic material will contain some nitrogen and phosphorus. Since this is irreversibly fixed in the refractory material, it is not subject to regeneration. The nitrogen and phosphorus bound in the algal refractory organic material will be referred to symbolically as N_{ra} and P_{ra}, respectively; and the nitrogen and phosphorus bound in the decomposer refractory organic material will be referred to as N_{rd} and P_{rd}, respectively.

Note that all the organic matter in heterogeneous biological systems initially free of organic carbon sources other than algae can be considered to be either algal organic material or organic material which originated from the decomposition and resynthesis of algal material. Thus the quantity of organic carbon, nitrogen, and phosphorus held in the total refractory organic material (including R_a and R_d) of a completely stabilized algal mass will affect the total quantity of oxygen required during aerobic decomposition and the quantity of nitrogen and phosphorus that can be regenerated. In other words, the refractory organic fraction of algal material is a major controlling factor in determining the extent of reversibility of Equation 1.

A complete mathematical description of the decomposition of algae with respect to time should consider all of the interactions shown in Figure 8. However, individual treatment of each decomposing population would quickly lead to an impractical mathematical expression. The following development is given with the objective of producing a practical expression which could be used to predict natural algal decay.

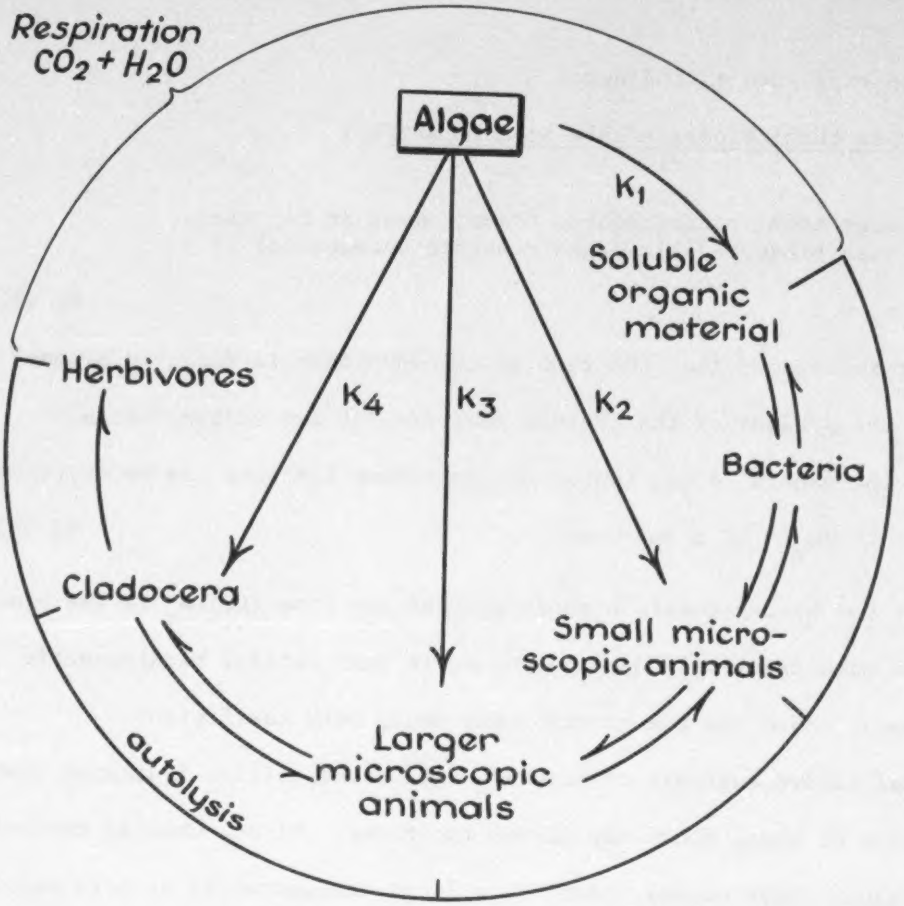
As shown in Figure 8 the change in algal mass with time at a given temperature may be considered to be a function of two main factors: 1) the algal mass, and 2) the decomposer populations. Thus the general function of the rate of decomposition of algae with time may be written:

$$\frac{-d\,(\text{algal mass})}{dt} = f\,(\text{algal mass, active decomposer mass}) \qquad \text{Eq (3)}$$

In order for any decomposer population to develop it must do so by the consumption of some organic food for energy and synthesis. Since algae are the food for this population it may be assumed that the active decomposer mass at any time is proportional to the algal material previously decomposed. Thus,

$$\frac{-d\,(\text{algal mass})}{dt} = f\,(\text{algal mass, algal mass decomposed}) \qquad \text{Eq (4)}$$

The active algal mass cannot presently be measured in a complex system. However, organic material resulting from the resynthesis of algal organic material can be considered to decompose in a manner similar to algal material. That is, the rate of change of any biodegradable organic particulate matter will depend on its mass as well as on the amount of material already decomposed. Thus the rate of change of the total biodegradable organic matter in an algal system with respect to


Respiration
$CO_2 + H_2O$
Algae
K_1
Soluble organic material
Bacteria
Small microscopic animals
Larger microscopic animals
Cladocera
Herbivores
K_4
K_3
K_2
autolysis

Small microscopic animals - rotifers, paramecium, stalked ciliates, and other protozoans.

Larger microscopic animals - worms

Cladocera - daphnia and other crustacea

Herbivores - fish and other algae eating animals

FIGURE 8. Processes involved in degradation of algae. Each population may revert back to another form either through some social interaction such as predation or through death and self destruction, i.e., lysis. These two processes are separated in the above figure.

time may be expressed as follows:

$$\frac{-d\ (\text{Mass total biodegradable organic matter})}{dt}$$

$$= f\ (\text{mass total biodegradable organic mass at any time, mass biodegradable organic matter decomposed})$$

$$= f\ (m, m_r) \qquad \text{Eq (5)}$$

If it is assumed that the rate of decomposition is directly proportional to the product of the organic mass present and that previously oxidized, the general algal system decomposition function can be written:

$$-\frac{dm}{dt} = K''\ mm_r = K''\ m\ (m_1 - m) \qquad \text{Eq (6)}$$

where m is the biodegradable organic mass at any time (t), m_r is the biodegradable mass previously decomposed, m_1 is some initial biodegradable organic mass, and K" is the second order decay rate coefficient.

A qualitative analysis of natural algal decomposition indicates that the kinetics of algal decay may assume two forms. First, when an explosive bloom of algae first occurs, there is a large concentration of pure algal cells, but few decomposers. In a short period of time the decomposers reach a significant population (as in Figure 9, case I) and the algae are then degraded. The second major type of decomposition occurs when algae grow more slowly in the presence of the decomposing populations (illustrated in Figure 9, case II). As the algae develop so does the population of decomposers. This is perhaps the more common case of algal growth in the natural environment. Subsequent decomposition of this mixed algal system can be seen to represent a limiting case for the general situation. That is, even when the algal mass is initially

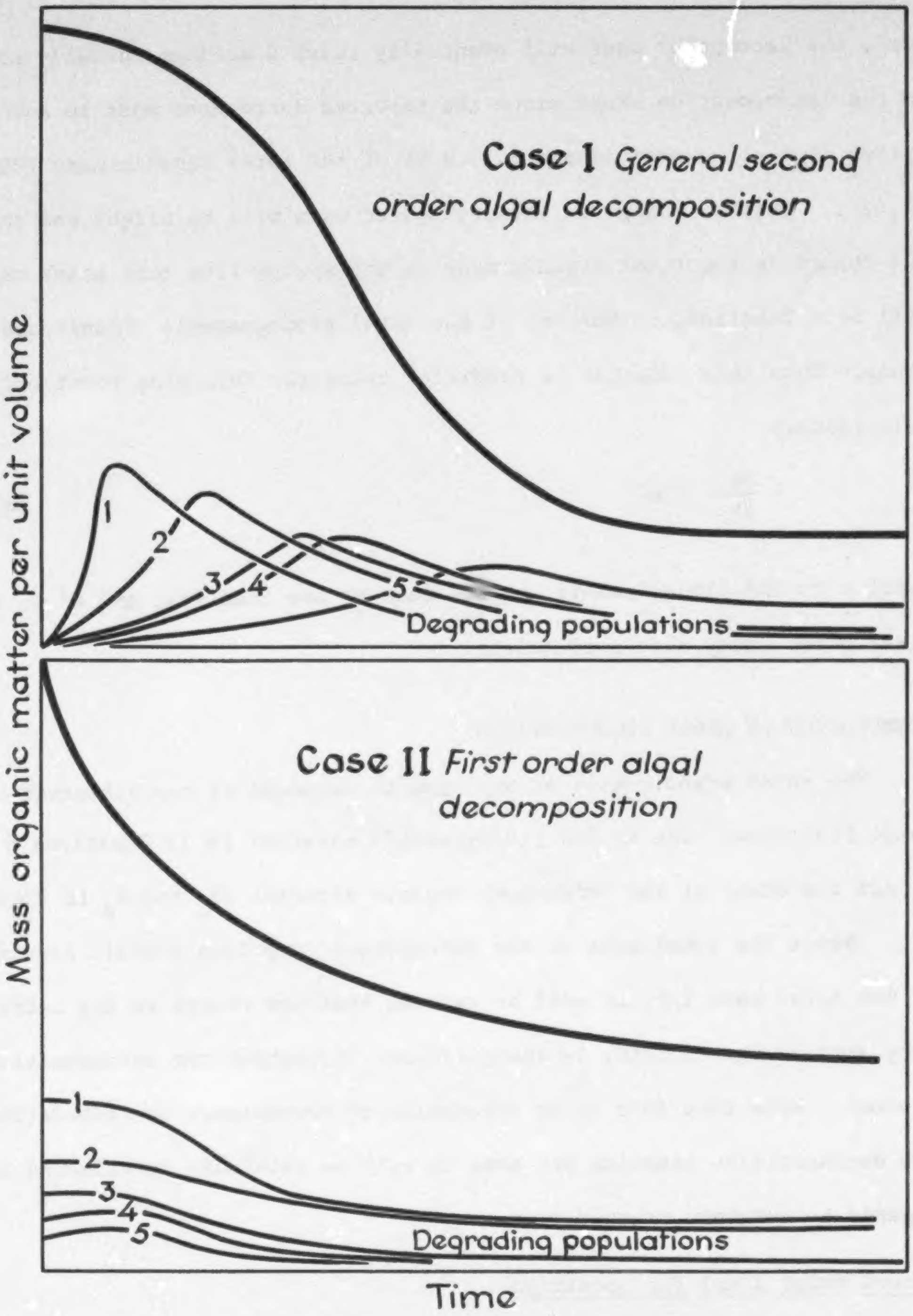


FIGURE 9. Illustration of the two extreme cases of natural algal decomposition; Case II is the more commonly observed and can be considered to be a limiting example of Case I.

pure, the decomposer mass will eventually reach a maximum (usually early in the decomposition stage since the reported decomposer mass in such a system does not exceed around 0.1 to 1% of the total organic mass (39,40, 41,42). Further change in the decomposer mass will be slight and thus the change in the total organic mass in the system from this point on will be a function, primarily, of the total biodegradable organic mass alone. Thus this case can be predicted using the following first order relationship:

$$-\frac{dm}{dt} = K'm \qquad \text{Eq (7)}$$

where m is the biodegradable organic mass at any time (t), and K' is the first order decay rate coefficient.

FORMULATION OF ALGAL DECOMPOSITION

The total organic mass at any time is composed of two different organic fractions. One is the biodegradable material (m in Equations 6 and 7) and the other is the refractory organic material (R_a and R_d in Figure 6). Since the total mass of the decomposers comprises a small fraction of the total mass (M), it will be assumed that the change in the refractory organic fraction (f) is insignificant throughout the decomposition process. Note that this is an assumption of convenience in formulating the decomposition kinetics but that it will be carefully reevaluated in regards to nutrient regeneration.

Second Order Algal Decomposition

In the general expression for algal decay where relatively pure cultures of algae grow and are seeded at some later date, the decay kinetics are hypothesized to follow second order kinetics according to Equation 6. This equation is undefined at time zero when M is set equal to M_o. It can be seen that this equation with this boundary condition would also be biologically unrealistic since it would indicate that the rate of change of the organic mass at time zero is zero. In any living system the rate of change in organic material is never zero but some finite number related to the respiration rate of the system. Also, if M were set equal to M_o, this would imply that the population of decomposers was equal to zero, in which case decomposition would never begin. In order to make the equation more biologically realistic, the following boundary conditions will thus be assumed: the initially measured biodegradable organic mass will be designated as M_o, and it will be assumed that the mass, M_1, is equal to M_o plus a certain value which is proportional to the initial mass of decomposers. Integrating Equation 6 between time equal zero and time "t," using the above boundary conditions and the total organic mass yields the following solution:

$$M = \frac{(M_1 - fM_o)Q}{1 + Q} + f\,M_o \qquad \text{Eq (8)}$$

where $$Q = \frac{M_o - fM_o}{M_1 - M_o}\, e^{-(M_1 - fM_o)K't}$$

and M is the total organic mass at any time, t, (days), f is the refractory organic fraction (refractory M/M_o), M_o is the total organic mass measured at t=0, M_1 is equal to M_o plus a constant to account for the initial seed of decomposing, and K" is the decay rate coefficient.

First Order Algal Decomposition

For all cases where bacteria and/or animals grow along with and in proportion to the growth of algae so that when decomposition begins there is initially a large population of decomposers, the decomposition kinetics may be approximated by a first order relationship. Using the total organic material of an algal system and the boundary limit that at t equals zero, the total measured organic material is M_o, integration of Equation 7 yields:

$$M = (M_o - fM_o)\ e^{-K't} + fM_o \qquad \text{Eq (9)}$$

where K' is the decay rate coefficient and the other variables are as defined previously.

By using properly defined rate coefficients (K' and K") and by estimating the refractory organic fraction of the organic material, the rate of decomposition of algae under all aerobic conditions may be predicted with Equations 8 and 9.

NITROGEN AND PHOSPHORUS REGENERATION

Nitrogen and phosphorus regeneration has been defined in Figure 1 as the transition of nitrogen or phosphorus in or on the algal cell or other algal derived organic material to any free soluble form. It is also essential to assume that no nitrogen or phosphorus is precipitated in an inorganic form or removed by a nonbiological process.

Regeneration of nitrogen and phosphorus from algal material is controlled by two overall factors: 1) availability (which is a measure of the ease with which nutrients are reverted to a soluble form), and 2) the concentration of the nitrogen and phosphorus in the organic mass. These two factors combine to make the regeneration of nutrients a very complex phenomenon. Using information on the consistency of algal composition and the energetics of bacterial and animal growth, the following hypothesis is given for defining the process of nitrogen and phosphorus regeneration from algae.

Quantity of Nitrogen and Phosphorus Regenerated

Of the total nitrogen and phosphorus held in the particulate organic material of an algal system, only a certain fraction will be regenerated. The nitrogen and phosphorus held in the refractory organic material (N_r and P_r, respectively) are not available. Thus the quantity of nitrogen and phosphorus which is available for regeneration can be expressed by the following equations:

$$N_b = N - N_r \qquad \text{Eq (10)}$$

$$P_b = P - P_r \qquad \text{Eq (11)}$$

when N_b, N and N_r refer to the nitrogen available for regeneration (mg/1), the nitrogen concentration in the particulate organic mass before decomposition begins, and the nitrogen bound in the refractory organic material, respectively (see Figure 5). The symbols are similar for phosphorus. Thus determination of N_r and P_r would enable the estimation of the quantity of nitrogen and phosphorus which would become available for further algal growth after the initial decomposition of the phytoplankton. The refractory organic material was indicated previously to be composed of two fractions: 1) algal refractory material - R_a, and 2) decomposer refractory organic material - R_d. The nitrogen and phosphorus held in these two fractions are designated N_{ra}, P_{ra} and N_{rd}, P_{rd}, respectively. Thus the total quantity of nitrogen and phosphorus unavailable for regeneration may be expressed as:

$$N_r = N_{rd} + N_{ra} \qquad \text{Eq (12)}$$

$$P_r = P_{rd} + P_{ra} \qquad \text{Eq (13)}$$

Information obtained from the literature can be used to estimate N_{rd} and P_{rd}. Bacteria and microscopic animals have been reported to be responsible for over ninety per cent of the energy change in natural ecosystems (59). Thus if the quantity of refractory organic material in the form of bacteria and microscopic animals, R_d, originating from algal decomposition and the concentration of nitrogen and phosphorus in R_d were known, this would allow estimates to be made of N_{rd} and P_{rd}.

Several researchers (17, 18, 158, 159) have considered the quantity of bacteria, or bacteria and microscopic animals which are synthesized under aerobic conditions during the decomposition of various organic substrates. Depending on the free energy of the substrate, the organisms making up the decomposers and many other factors, it was reported that the quantity of organic material initially resynthesized by the decomposers varied between 20 and 90 per cent for a wide variety of conditions, with a usual value of around fifty per cent. From this the quantity of decomposers which are formed during aerobic algal decomposition may be estimated by the following expression:

$$(B_{d\ max} + R_d) = 0.5\ B \qquad \text{Eq (14)}$$

where ($B_{d\ max} + R_d$) is the total decomposer organic material originating from complete aerobic stabilization of algal mass, B (as shown in Figure 7).

Washington and Symons (18) and Kuntz and Forney (17) in studies specifically designed to determine R_d of complex bacterial and microscopic animal systems (activated sludge) reported a value equal to between 20 and 25 per cent of the total decomposer mass. Thus it will be estimated that:

$$R_d = 0.20\ (B_{d\ max} + R_d) \qquad \text{Eq (15)}$$

Symons and McKinney (160) noted that the nitrogen concentration in activated sludge varied from 10 to 2 per cent when subjected to extreme nitrogen starvation conditions. It is possible that starvation conditions might affect N_{rd} and P_{rd}. However, Washington and Symons (18) have grown activated sludge systems in conventional and completely mixed units on several different substrates and have noted that the main cellular components were quite uniform. They also determined R_d for these systems. Table 8 compares the composition of actively growing activated sludge to the refractory organic material composition.

Table 8. Composition in per cent of organic portion of complex mixtures of bacteria and microscopic animals (activated sludge) fed different substrates (18).

I. Composition of actively growing activated sludge.

	Per cent Volatile Suspended Solids			
Substrate	Protein	Fat	Carbohydrate	Nitrogen
Acetate	51.1	5.9	41.7	8.1
Glucose	52.9	6.6	40.1	8.4
Glycine	69.8	6.2	24.0	11.1

II. Composition of refractory organic material.

Substrate	Protein	Fat	Carbohydrate	Nitrogen
Acetate	39.0	5.0	56.0	6.2
Glucose	43.0	6.0	51.0	6.9
Glycine	47.0	6.0	47.0	7.6

The significance of the above results (18) to this study is that the refractory fraction of all activated sludge systems was very similar and not a great deal different than the actively growing composition. The final nitrogen content of the refractory organic material was reported to have a maximum variation (all data not summarized in Table 8) of 3.56 to 10.7 per cent (volatile suspended solids), with an overall average of 6.7 per cent. It will therefore be assumed in this study that the final nitrogen concentration in the decomposer refractory organic material is 7 per cent of the volatile suspended solids.

Less is known regarding the phosphorus content of R_d. However, since it has been shown that there is relatively little difference between the nitrogen content of the actively growing decomposers and that measured in R_d (see Table 8), it seems reasonable to assume that the phosphorus concentration in R_d will be similar to that in actively growing systems. The phosphorus concentration of complex bacterial and microscopic animal systems (activated sludge) has been reported to vary between 1 and 4% (VSS) with an average of around 2 per cent (161,163,164) Since the phosphorus requirement of bacteria is often noted as being 1/5 to 1/7 that of the nitrogen requirement (158,160,165,168), it will be estimated that the phosphorus concentration is: $P_{rd} = 1/5 \; (N_{rd})$ or 1.4 per cent phosphorus (on a volatile suspended solids basis).

Using the information previously presented and the relationship that one milligram of volatile suspended solids has a COD of 1.42 milligrams of oxygen (167) for most bacterial and animal organic material, the following equations can be used to estimate the nitrogen and phosphorus bound in the decomposer refractory organic material (R_d) which originates during algal decomposition.

$$N_{rd} = 0.5 \frac{(M_o - fM_o)}{1.42} \times 0.20 \times 0.07 = 0.00494\ (M_o - fM_o) = 0.0050\ (M_o - fM_o) \qquad \text{Eq (16)}$$

$$P_{rd} = 0.5 \times \frac{(M_o - fM_o)}{1.42} \times 0.20 \times \frac{0.07}{5} = 0.000988(M_o - fM_o) = 0.0010(M_o - fM_o) \qquad \text{Eq (17)}$$

where N_{rd} and P_{rd} are the nitrogen and phosphorus concentrations in R_d in mg/l, respectively, and $(M_o - fM_o)$ is the initial total biodegradable COD (mg/l) of the algal mass (measured by the difference between the initial total COD and the total COD after complete aerobic decomposition).

The quantity of nitrogen and phosphorus held in the refractory algal material (N_{ra}, P_{ra}) is complicated by the variability of the nitrogen and phosphorus in the algal cell during growth (as illustrated with nitrogen in Figure 6). Excess nutrients (N_L) which are often present in young algal cells disappear in the absence of extracellular nitrogen, leaving all nutrients combined in a more permanent form in the algal organic material (as represented by the minimum nitrogen concentration, N_m). Goldberg _et al._ (98) showed that the concentration of phosphorus in the diatom, _Asterionella japonica_, decreased three to four fold as growth depleted the available phosphorus in solution from 0.1 to 0.016 mg/l phosphorus. With diatom cells with high phosphorus concentration, it was reported that as much as fifty per cent of the phosphorus was easily washed from the diatom cells with phosphate free water similar to that in which the diatoms were growing. However, cells which were allowed to grow until 90 per cent of the available substrate phosphorus had been utilized resulted in the production of diatoms with the concentration of phosphorus in the cell close to the minimum requirement. No regeneration of phosphorus was noted in a study in which the diatoms with the minimum phosphorus concentration were allowed to decompose in the presence of bacteria for three weeks in the dark. Unfortunately all the phosphorus concentrations determined by Goldberg *et al*. (98) were reported on a cell concentration basis and therefore are not available on a more applicable basis such as volatile dry weight.

A review of the phosphorus concentration in algae growing at different rates indicates that the general phenomenon reported by Goldberg *et al*. (98) for the regeneration of phosphorus is probably true for nitrogen. That is, as long as there is excess nitrogen and phosphorus available in the medium, the algal cell will probably store a certain quantity in the cell (ranging up to 16 and 5 per cent of the algal dry weight for nitrogen and phosphorus respectively - N_L and P_L as shown in Figure 6). Fifty per cent or more of these nutrients will be highly available for regeneration. As algal growth progresses the excess nutrients in the media will be eventually depleted and as this occurs the cell will call first upon the luxurious stores of nutrients (N_L and P_L) for continued growth and then upon the normally required nutrients (146,168). Growth will continue at a slower and slower rate until either N_m and P_m or both of these nutrient concentrations reach the minimum concentration, after which growth will cease. As the nutrient concentrations progress from the luxurious to the minimum requirements, the quantity of nutrients available for regeneration will decrease.

It is reasonable to assume that for practical purposes two different estimates of N_{ra} and P_{ra} must be made and that the dividing point between "young" algal cells and "older" cells will be somewhere between cells having the concentration of nutrients corresponding to the "normal requirements" and those with the "minimum" concentration (see "Y" and "O" cell designation in Figure 6).

The rate of absorption of phosphorus by the diatom, Nitzschia closterium has been reported to be directly dependent on the concentration of both phosphorus and nitrogen in the medium, but nitrogen absorption was independent of the phosphorus concentration (101). This indicates that the dividing point between the two different types of algal cells is more dependent on the nitrogen than the phosphorus concentration in the algal cell. Some researchers have reported that algae do not have an appreciable growth rate unless the nitrogen concentration is equal to or greater than 3 to 4% (47,138,169). Algae growing under limited nitrogen and phosphorus conditions have also been reported to contain between 3 and 5% nitrogen (138,169). This indicates that the division between "young" cells growing at unlimited rates and "older" cells with restricted growth is that point at which the nitrogen content equals 3% (on volatile suspended solids basis).

Case 1. Young algal cultures. When algal systems have a nitrogen concentration greater than 3 per cent (VSS basis), and/or when the concentration of soluble nitrogen and phosphorus is not limiting, then it is postulated that N_{ra} and P_{ra} may be estimated from the concentration of nitrogen and phosphorus which just begin to limit the rate of growth (as shown in Figure 6). This value is taken as 3% (VSS basis) for nitrogen and 0.3% for phosphorus, since phosphorus requirements of algae are reported as being approximately one tenth the nitrogen requirement (141). Since R_d seldom amounts to more than a few per cent of R*, an estimate of R or of the total refractory organic material present may be used as an estimate of R_a. Thus the following equations may be used to approximate N_{ra} and P_{ra} in "young" algal cells.

$$N_{ra} = \frac{0.03}{1.42} R' = 0.021 R' \qquad N \geq 0.03 \text{ (VSS)} \qquad \text{Eq (18)}$$

$$P_{ra} = \frac{0.003}{1.42} R' = 0.0021 R' \qquad P \geq 0.003 \text{ (VSS)} \qquad \text{Eq (19)}$$

where R' is the sum of R'_a and R'_d expressed as the COD of the suspended solids and measured after biological decomposition has ceased. Note that in Equations 18 and 19 it is necessary to use only the suspended solids COD to estimate R since when the final total refractory organic material is low (10 to 30 mg/l COD) the soluble refractory COD may form a significant fraction of the total.

Case II. Older algal cultures. When the algal system has a nitrogen concentration less than three per cent (VSS basis) it is postulated that the per cent of nitrogen and phosphorus in the total mass before decomposition starts is approximately the same as that remaining in the refractory organic material after decomposition is complete. Equations for predicting N_{ra} and P_{ra} in "older" algal systems would then be as follows:

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*For example, if an algal system has a biodegradable fraction (B) = 60%, then the decomponents resynthesized on this would be 0.5(60) = 30% of which one fifth or 6% would be R_d. Since R_a + R = 40%, a fair approximation to R_a would be given by a measurement of the total refractory organic material ($R_a + R_d = R$).

$$N_{ra} = \frac{N'}{100} (R') = f(N) \qquad N < 0.03 \text{ (VSS)} \qquad \text{Eq (20)}$$

$$P_{ra} = \frac{P'}{100} (R') = f(P) \qquad P < 0.003 \text{ (VSS)} \qquad \text{Eq (21)}$$

Where N' and P' are the initial nitrogen and phosphorus concentrations in per cent (COD basis), R' is the final refractory suspended solids COD mg/l, f = Final refractory suspended solids concentration/Initial total suspended solids concentration, and N and P are the initial particulate organic nitrogen and phosphorus in mg/l.

In summary it can be seen that Nr and Pr can be estimated for all algal systsms. Recalling that the total quantity of nitrogen and phosphorus which is available for regeneration is:

$$N_b = N - N_r \qquad \text{Eq (10)}$$

$$P_b = P - P_r \qquad \text{Eq (11)}$$

Since N and P are the initially measured concentrations of nitrogen and phosphorus in the algal material, an estimate of N_r and P_r would allow the calculation of the quantity of nitrogen and phosphorus which could be regenerated.

N_r and P_r are composed of two fractions, one in the decomposers and one in the algae, i.e.:

$$N_r = N_{rd} + N_{ra} \qquad \text{Eq (12)}$$

$$P_r = P_{rd} + P_{ra} \qquad \text{Eq (13)}$$

From an estimate of the quantity of decomposers which forms during the aerobic decomposition of an organic substrate, the refractory fraction of this material, and the nitrogen and phosphorus which is bound in the decomposer refractory organic material can be found:

$$N_{rd} = 0.005\ (M_o - fM_o) \qquad \text{Eq (16)}$$

$$P_{rd} = 0.0010\ (M_o - fM_o) \qquad \text{Eq (17)}$$

Depending on the algal age and therefore the concentration of nitrogen in the algal cell N_{ra} and P_{ra} may be estimated by one of the following sets of equations.

Case 1. Young algal cells with nitrogen content greater than 3 per cent (VSS).

$$N_{ra} = 0.021\ R' \qquad N \geq 0.03\ (\text{VSS}) \qquad \text{Eq (18)}$$

$$P_{ra} = 0.0021\ R' \qquad P \geq 0.003\ (\text{VSS}) \qquad \text{Eq (19)}$$

Case II. Older algal cells with a nitrogen content less than 3 per cent (VSS).

$$N_{ra} = f(N) \qquad N < 0.03\ (\text{VSS}) \qquad \text{Eq (20)}$$

$$P_{ra} = f(P) \qquad P < 0.003\ (\text{VSS}) \qquad \text{Eq (21)}$$

Thus by using the above equations N_{rd}, N_{ra}, P_{rd} may be estimated for all algal systems thus allowing the calculation of the quantity of nitrogen and phosphorus which will be regenerated, N_b, P_b. Note that all these equations depend on a valid determination of the refractory organic material in algal systems.

Rate of Nitrogen and Phosphorus Regeneration

To completely define nutrient regeneration both the rate of regeneration and the total quantity of nutrients to be regenerated must be known. In the previous section an hypothesis has been proposed which can be used to predict the quantity of nitrogen and phosphorus which can be regenerated during complete aerobic decomposition of algae. This relationship indicates whether regeneration is to be expected but does not allow prediction of the rate at which it occurs.

The kinetics of nitrogen and phosphorus regeneration depends primarily on four major variables: 1) the nitrogen and phosphorus concentration in solution, 2) the available nitrogen and phosphorus in the algal material, 3) the quantity of organic matter to be decomposed, and 4) the rate of decomposition of the organic material. Because of the complexities of the biological action occurring in Action zones A and B of Figure 5 due to the influence of the above factors, no attempt will be made to quantitatively define the kinetics in these two zones. However, a knowledge of the decomposition kinetics help in understandng nitrogen and phosphorus regeneration.

As noted earlier the rate controlling step in algal decomposition is the enzymatic hydrolysis of algal material. This step is so slow that the resulting mass of decomposition does not usually exceed 0.1 to 1 per cent of the total organic mass during active decompostion and seldom exceeds 10 per cent in the refractory organic materil after decomposition is complete. The maximum population of bacteia usually occurs during the first few days of algal decomposition (2029). Thus it can be seen that the changes in the nitrogen and phosphorus components of an algal system in Zones A and B (Figure 5) result from the release of nitrogen and phosphorus from the algal material and the resulting reabsorption during growth of the decomposer population. The initiation of nutrient regeneration, i.e., the beginning of Zone C (Figure 5) occurs when the quantity of nutrients released exceeds the requirements of the decomposer mass. Recalling Equations 20 and 21 note that the final concentration of nitrogen and phosphorus in the algal material can be estimated by assuming that the per cent organic material remaining is an approximate of the per cent of the initial particulate nitrogen and phosphorus which remains in the refractory organic material. This same reasoning can be applied to nitrogen and phosphorus regeneration in Zone C of Figure 5. That is, after an equilibrium decomposer mass has developed and nutrient regeneration is initiated the regeneration of nitrogen and phosphorus will be dependent upon the reduction in carbonaceous organic material. Since the mass of decomposers changes very little an equivalent amount of nutrients released by the algal material will be released by the decomposers. For example, if the reduction in organic mass measured after the beginning of Zone C is 10 per cent, then the reduction in particulate nitrogen and phosphorus will be approximately 10 per cent. Since for every unit change in organic carbon there will then be a proportional change in the particulate nitrogen and phosphorus, the regeneration kinetics in Zone C can be assumed to be proportional to the kinetics of organic material decomposition in Zone C, i.e.:

$$\frac{d\,(N \text{ or } P)}{dt} = \alpha \frac{d\,M}{dt} \qquad \text{Eq (22)}$$

where d(N or P)/dt is the rate of change of particulate nitrogen and phosphorus, dM/dt represents the rate of decomposition of the algal mass, and α is a proportionality constant.

IV. EXPERIMENTAL PROCEDURES

In order to fulfill the objectives of this research it was necessary to determine the significance of many variables of algal decomposition and nutrient regeneration. To determine whether generalizations could be made regarding growth and decay of natural algal cultures a variety of natural water samples ranging from ocean water to mountain spring water, and from pure water to highly polluted water, were collected for study. The available information did not indicate whether algal growth would occur in all waters, the extent of the growth which might be expected or the relationship between chemical composition of the water and growth. After establishing the relationship between heterogeneous algal growth and decay in natural waters, more closely defined experiments were conducted using defined media and pure algal cultures to explain the mechanisms controlling the cycles of interest.

Because of the wide ranging objectives of this study a number of experimental approaches were used to obtain the results. Although all experiments were similar in many respects, each one differed slightly in order to allow measurement of the desired variable.

There were seven general experiments in this study. The following list includes the name of each of these experiments and the general objectives sought.

Table 9. Summary of Experiments and Objectives

EXPERIMENT	GENERAL OBJECTIVES
I. High Nutrient Level Algal growth and decay	To determine the biodegradability of algae, to determine the general range of variables to expect, and to test experimental design.
II. Algal growth in sewage treatment plant effluent	To determine the refractory organic fraction of a series of easily obtained algal cultures.
III. Intensive Simulated Natural Algal Studies	
A. Major experiments on six different types of water	To fulfill all objectives as stated in the initial part of this study.
B. Short Studies	To determine the relative effect of several variables on the decomposition process.
1. Fractionated algae versus live algae	To determine the effect of live versus dead algae on the decomposition process.
2. Anoxic conditions	To determine the effect of an anoxic environment on algal decomposition.
3. 30 minutes of light	To determine the effect of short periods of light on algal decomposition.
IV. Young algal cell studies	To substantiate data obtained from young cell decomposition in previous experiment.
V. Pure Culture	To determine the effect of temperature on algal decay, and to supplement information from previous experience.
VI. Chlorophyll study	To correlate oxygen utilization with the chlorophyll content of the algal system.
VII. Nutrient supplementation studies	To determine the effect of added nutrients and organic matter on the rate and extent of decomposition.

The detailed description of each of these experiments will be given along with the data in the results section.

Common Equipment and Experimental Conditions

The experimental apparatus used in the growth and decomposition experiments is illustrated in Figure 10. All decomposition studies were carried out under similar physical conditions, but the growth conditions in Experiment II were different from all others (see ref. 189). In all growth experiments, except Experiment II twenty liter pyrex carboys were used as algal system containers. These carboys were fitted with rubber two hole stoppers which had two glass tubes placed in them. The aeration inlet tube extended to within one half inch of the bottom of the containers while the outlet glass tube extended about one half inch below the rubber stopper. These carboys were initially filled with 18 liters of appropriate sample water and exposed to diurnal light in a constant temperature room held at 20° ± 0.5°C. During the course of the study no more than six algal growth vessels were used at one time. When 16 liters of samples had been removed from the growth vessels for subsequent decomposition studies, the remaining samples in Experiment III and IV were transferred to a 4 liter pyrex glass growth vessel and placed in other growth vessels similar to that shown in Figure 10. By using this method of rotation the long term growth studies carried on in Experiments III and IV could be continued while the main growth apparatus was prepared for Experiments V and VI, successively.

At predetermined time periods "branch" samples were taken from the growth vessels and placed in decomposition vessels. In all cases, except in Experiment III (B-3), the algal samples were placed in complete dark-

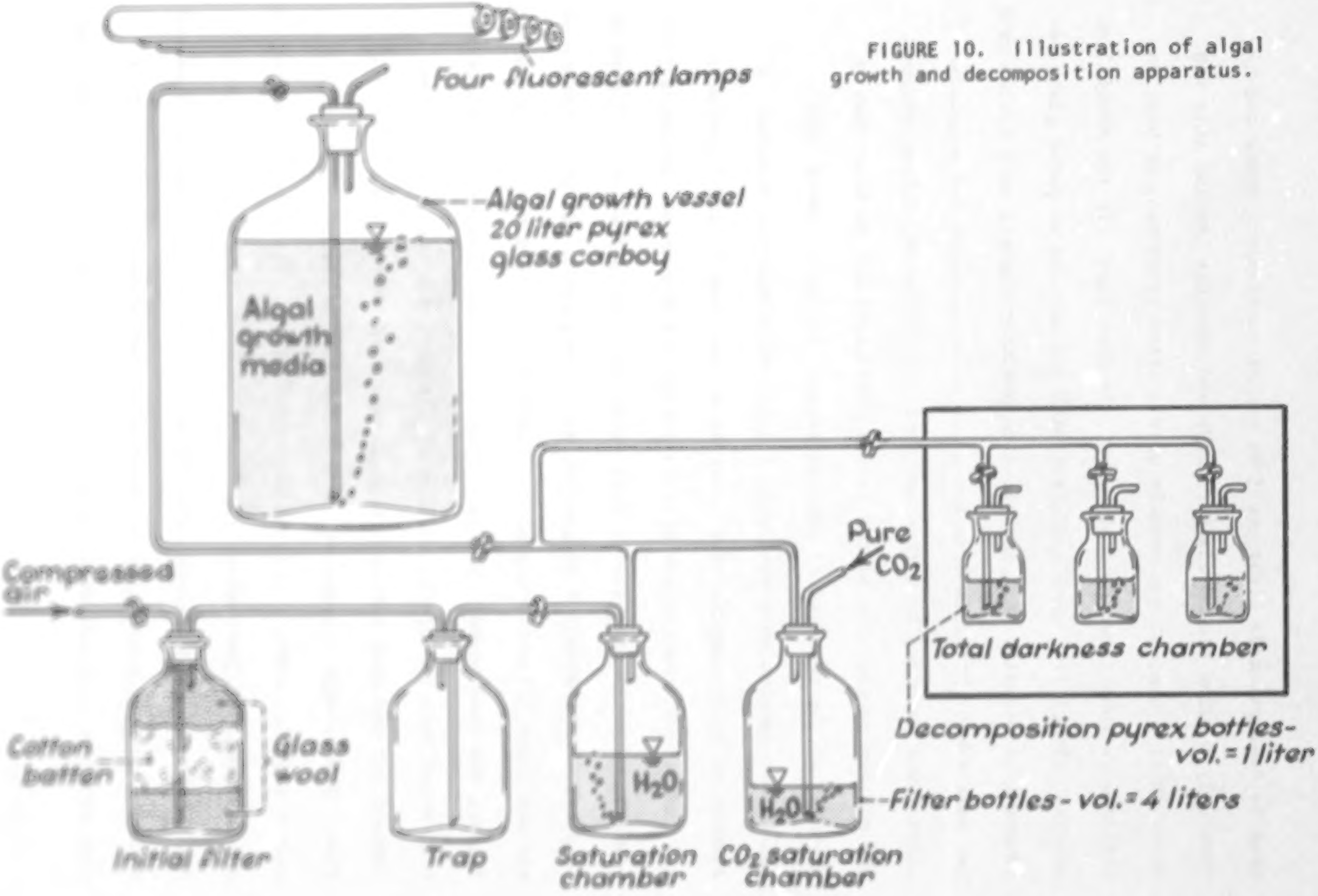
Four fluorescent lamps
Algal growth vessel
20 liter pyrex
glass carboy
Algal growth media
Compressed air
Cotton batten
Glass wool
Initial filter
Trap
Saturation chamber
H_2O
CO_2 saturation chamber
Pure CO_2
Total darkness chamber
Decomposition pyrex bottles-
vol.=1 liter
Filter bottles - vol.=4 liters

FIGURE 10. Illustration of algal growth and decomposition apparatus.

ness in cabinets made light proof by use of overlapping doors and by carefully sealing around all air lines and openings leading into the area. Most decomposition vessels were 1 liter pyrex reagent bottles fitted with rubber two hole stoppers. In Experiment III the short term studies (B-1,2,3) were conducted with 500 and 250 ml pyrex glass decomposition vessels. All aerobic decomposition vessels were fitted with two hole rubber stoppers with two glass tubes provided for aeration. The inlet tubes extended to within one half inch below the rubber stopper. The maximum sample size placed in the containers was no more than 90 per cent of the capacity of the container. In most cases 900 milliliter samples were used with 1 liter decomposition vessels. A maximum of fifty samples were aerating at one time during Experiment III. An air-CO_2 mixture was used to aerate all growth and decomposition samples. Several precautions were taken with the apparatus in Figure 10 to prevent contamination of samples with the gaseous mixture. Compressed air was first filtered through a 4 liter packed glass wool-cotton batten filter, and then passed through a trap to prevent passage of the saturation water into the filter in case of a negative pressure. The compressed air was then bubbled through deionized water to saturate the flow. Pure carbon dioxide was introduced into a similar four liter container and also bubbled through deionized water. The water saturated air and CO_2 was then combined at a "T" intersection and then distributed to the growth and decomposition vessels by rubber tubing. The CO_2 flow was adjusted to give a quantity of about 1% carbon dioxide in air. This flow rate was qualitatively checked and adjusted by noting the bubbling rate of the two separate water saturation containers in the aeration set-up (as shown in Figure 10). Approximately 1% carbon dioxide in air was found to maintain a constant pH between 6.8 and 7.3 in all samples studied. The flow rate of the air-CO_2 mixture going into each vessel was controlled by a screw clamp on the inlet tubing. In the simulated natural algal studies (in Experiment III) the flow into the growth vessels was also used to mix the sample and thus was adjusted to approximately 300 bubbles per minute. However the air-CO_2 flow going into all decomposition vessels was much slower. Since these samples were kept in the dark as much as possible it was necessary to adjust the bubble rate by some means other than visually. It was found that a standard medical stethescope could be used for this purpose. The aeration rate of around 100 bubbles per minute (1/4 inch ID glass tubing) appeared to cause a gentle rolling action in most 900 ml decomposition samples and thus prevented the accumulation of large clumps on the bottom and insured a fairly uniform distribution of dissolved oxygen throughout the samples. The air flow used in the pure culture growth studies in Experiments V and VI was similar to that in Experiment III but an added precaution was taken to maintain uniform dispersion of the algae by providing each vessel with a magnetic stirrer. It has been reported that magnetic stirring of diatoms crushed the cells (101), but this was not found to occur in this experiment. Apparently the slow speed of the two inch (3/8 inch diameter) polyethylene stirring bar, and the large volume of sample, prevented this factor from being of significance. Several precautions were taken with the aeration apparatus in Experiments V and VI to protect the growth and decomposition samples from bacterial contamination. An additional 12-inch long, 2-inch diameter cotton-batten filter was added after the air had been mixed with the CO_2 and a combination cotton and glass wool plug was added to the inlet and the outlet tube of each growth and decomposition vessel. These precautions appeared to be adequate to maintain pure algal samples for a period of up to one hundred days.

All tests, except the low temperature study in Experiment were carried out in temperature controlled conditions in constant temperature walk-in incubators. The temperature variations of these rooms were less than ± 1/2°C for any period of time greater than 15 minutes. The exception was a modified refrigerator used in the low temperature study, where the variation of liquid temperature was found to be less than a degree at 4°C. Several unexpected problems were met in the aeration of samples at temperatures much higher or lower than room temperature. In the 35°C decomposition studies the evaporation rate was much higher than in the other samples and thus had to be carefully monitored to avoid significant changes of sample liquid volume. At four degrees centigrade it was found that the opposite effect occurred, i.e., condensation of water from the aeration mixture took place. Thus it was necessary to place a condensation trap in the air line inside the 4°C decomposition incubation area.

All algal growth conditions were similar in this study except with Experiment II. Algae were exposed to automatically controlled diurnal light in the 20°C constant temperature room. Diurnal illumination (12 hours of light and 12 hours of darkness) was provided by using 4 forty watt Ampex "Daylight" fluorescent bulbs mounted directly over the samples. The light intensity was found to be 1600 foot-candles as measured three inches below the bulbs (as measured by a Weston Light meter). The initial surface of the algal sample was six inches below the bulbs with the bottom of the containers about 17 inches below the bulbs. The light intensity in Experiment III (B-3) was 300 foot-candles measured at the sample surface.

The intensity of color development for all color dependent tests was measured with either a Coleman Junior Spectrophotometer, Model GA or a Beckman Spectrophotometer, Model D.B. Sensitivity of both models appeared to be comparable for the tests considered. The pH was measured using a Beckman zeromatic pH meter. A Beckman Infra-red carboneceous analyzer was used for all carbon measurements.

General Experimental Procedure

Because many of the nitrogen, phosphorus and carbon measurements were being conducted in the region approaching the minimum accurately detectable quantities it was important that extra precautions be taken with all procedures. Particular attention was paid to cleaning the glassware. The general procedure was to clean all glassware after using several times with soap ("Labtone") and water, and then with Standard Methods (170) chromic-acid cleaning solution. In some cases to remove extra stubborn stains or salt accumulation a fluoric acid cleaning solution was used. This solution consisted of 10 gm of sodium floride and 10 ml of sulfuric acid in 1 liter deionized water. The exposure of glass surfaces to this final cleaning solution for a period of 30 seconds was found to remove all organics and inorganics. However, longer exposure would etch the glass.

Perhaps the single most important procedure was the sampling method. In order to obtain a representative sample from the algal system vigorous mixing was employed while the samples were withdrawn with a pipette. In most cases this meant removing 50 to 150 ml with a pipette. The large 18 liter growth vessels were removed from the growth apparatus and shaken. The tops of the growth vessels were covered with solid rubber stopper, each wrapped with a plastic membrane ("Saran Wrap") prior to shaking to prevent contamination and loss of the sample. While the sample was still swirling a pipette was thrust 3 to 4 inches below the surface of the algal culture and about midway between the center of the container and the edge. By drawing up the sample slowly in the pipette it was felt that a fairly representative sample was obtained. In all cases where organic matter accumulated on the sides of the container and where vigorous mixing could not remove it, a rubber policeman was used to scrape the sides. Fortunately only a few samples had algae which showed a tendency to grow on the sides of the containers and with these few samples this characteristic was lost within several weeks in the dark decomposition vessels.

This same general sampling procedure was also followed with most of the decomposition samples. However, since the vessels were much smaller, it was easier to shake the samples much more vigorously. In some cases where large clumps had formed it was necessary to use a magnetic stirrer over a period of hours to achieve a dispersion of particles throughout the culture. In all cases one large sample was taken from the main culture and then all tests were run with this sample.

With the pure culture samples it was necessary to sterilize all sampling equipment beforehand. It was found that large samples could be maintained sterile and transferred by using a sterile siphon. In the decomposition studies only 8 to 10 samples could be taken because of the culture size. About 100 ml was required to analyze for nitrogen, phosphorus, and carbon. Thus the maximum number of analyses completed for most of the decomposition studies was 8 or 10. With Experiment V 6 or 8 samples were taken for analysis, while with the long term growth studies of Experiment III.30 to 40 were taken.

Before each sampling period, evaporation was compensated for by the addition of deionized water. For sample sizes greater than 4000 ml the evaporation was compensated for by filling to a volumetric mark on the outside of the containers, while for samples smaller the evaporation was corrected for on a weight basis. The saturation of the air-CO_2 flow with water prevented the bulk of evaporation, so that the evaporation was usually less than 0.5 ml per day at 20°C. However, this rate increased to 2 or 3 ml per day at 35°C and decreased to close to zero at 4°C. When the decomposing algal samples reached apparent stabilization the normal bubbling rate was decreased from about 100 bubbles per minute to 15 or 20 to prevent excessive evaporation.

ANALYTICAL TECHNIQUES

To adequately define the effect of algal decomposition on the oxygen, carbon, nitrogen and phosphorus resources of natural waters it would be necessary to measure many chemical and physical parameters. In order to simplify the situation in this study most of the major physical parameters such as lighting, temperature, and pH were held constant for any particular experiment. As algal growth and decomposition proceeded all the components shown in Figure I were measured either directly or indirectly in one or all experiments in this study. The chemical oxygen demand (COD) test was used as a measure of the total and particulate organic material in nearly all cases. At most of the sampling periods the following forms of nitrogen and phosphorus were measured: Nitrogen - total organic, organic, organic particulate, ammonia, nitrite, and nitrate; phosphorus - total phosphorus, total phosphorus of the filtrate, and ortho phosphorus. The total organic carbon, filtrate organic carbon, and total suspended solids were measured nearly as frequently as COD. Analyses that were made less frequently for shorter definitive studies included such tests as the BOD_5, Warburg oxygen uptake measurements, electrolysis BOD, chlorophyll content, lipids determination (total chloroform extractables), and volatile suspended solids.

Unless otherwise noted, the analytical techniques used in this investigation were obtained from Standard Methods for Examination of Water and Wastewater, twelfth edition, 1965, published jointly by the American Public Health Association, the American Water Works Association and the Water Pollution Control Federation (170). Those tests which differ from the tests described in Standard Methods will be discussed in the following paragraphs along with the testing errors involved with this study.

Suspended and Volatile Solids

Suspended volatile solids measurements was made using glass fiber filters (Whatman's glass fiber filter, GF/c, 4.54 cm diameter) and a Millipore filter holder in the manner described by Wyckoff (171). In nearly all cases with suspended solids ranging from less than 10 to over 2000 mg/l the filtrate always appeared crystal clear indicating a very high efficiency of removal of particulate matter with the algal systems studied. Note that this is an important consideration in obtaining accurate data for the nutrient regeneration hypothesis postulated since any material which ends up in the filtrate is, by definition, soluble and therefore regenerated nutrients.

Only in the case where the sample water had a high salt content, such as San Francisco Estuary water, was any distilled water used for washing the suspended material on the filter pads. Comparisons of washed and unwashed samples of fresh river and lake water indicated that the salts retained by the glass pads were insignificant and not measurable in most cases. Another reason for not washing most suspended solid samples was because vigorous washing was shown to wash some of the organic material out of the phytoplankton cells. Since the solids retained on the filter and the filtrate were used in further tests all harsh treatment of the cells was avoided. Only with one sample in one run did the filtrate appear different than the supernatant liquor of the sample after the algae had settled. In this one case it appeared that some of the chlorophyll was released into solution as the cells were pressed against the pad by vacuum pressure. In most tests a minimum of two and sometimes four samples were filtered onto the glass pads. Usually two samples were dried for at least an hour at 103°C and then weighed to determine the suspended solids. These two filters were then used to determine the particulate COD and particulate organic nitrogen. In tests where it was anticipated that a large fraction of the particulate nitrogen might be in an easily solubilized state (as with luxurious nitrogen storage) total Kjeldahl nitrogen was determined using unwashed and undried, but filtered particulate material. A fourth filter was sometimes used to collect suspended solids in order to measure the chlorophyll concentration. This filter was also unwashed and undried, and it was also submerged in a film of deionized water until the chlorophyll analysis could be completed (not more than several hours after filtration).

Total and Particulate COD

The chemical oxygen demand test (COD) was similar to that described in Standard Methods (170) except for the following changes. In order to expand the accurate range of the standard test, three different normalities of potassium dichromate were used, together with a matching normality of ferrous ammonium sulfate so that a constant titrant volume (approximately 20 ml for blanks) would be maintained. The following table summarizes these changes:

Table 10. Concentration of reagents used in the COD test

Normality of potassium dichromate	Approx. normality of ferrous ammonium sulfate	Approx. maximum COD range (using 20 ml sample size)
0.025	0.012	80
0.0833	0.040	300
0.250	0.100	800

A silver sulfate catalyst was used in this test. Mercuric sulfate was also added to inhibit chloride interference. Tests showed that with concentrations of chloride such as found in San Pablo Bay of the San Francisco Estuary, the mercuric sulfate additive recommended by Standard Methods (170) effectively blocked interference from chloride oxidation. However with sea water samples (Monterey Bay) this modification was found not to consistently apply even when sufficient mercuric sulfate was added. Therefore the COD test with all sea water samples was used only on the suspended solids.

Standard Methods (170) reports a standard deviation of ± 8.2 per cent using reagent grade glucose. The mean was found to be 92 per cent of the theoretical oxygen demand. For most organic compounds the oxidation has been reported to be usually greater than 95 per cent of the theoretical value (172, 173). The error introduced by using undiluted reagents in the COD test for a wide range of COD values is illustrated by the following test. COD reagents with a maximum range of 800 mg/l were used to measure the COD of 20 duplicate samples from an algal system with a COD of approximately 64 mg/l. The standard deviation was found to be 14.5% of the average, or almost twice the standard deviation when employing a more appropriate strength of reagents. In this study it may be assumed that the accuracy of the test employing three dilutions of reagents is similar to that reported by Standard Methods (170). This is true only when precautions are taken to use specially cleaned glassware and sampling techniques that introduce a minimum of error at extremely low COD values.

The minimum variability of the COD test was determined by using a series of blanks and 0.0250 N potassium dichromate. The average COD added by reagents and possible contamination from the glassware (condensers, etc.) was found to be 1.68 mg/l with a standard deviation of 1.4 mg/l.

The COD of the particulate material was determined by analyzing solids retained on a glass fiber filter along with the filter itself. This procedure added another error to the COD test since the glass pads had a COD which varied from filter to filter. In one series of tests using 0.025N $K_2Cr_2O_7$ the average COD of the filter pad is 20 ml of water was found to be 9.2 mg/l with a standard deviation of 1 mg/l (or 0.0184 mg ± 0.002 per filter pad). But a series of tests using glass filter pads from many different boxes over a period of two years using all three concentrations of reagents (given in Table 10 gave an average COD in 20 ml water of 15.5 mg/l with a standard deviation of 9.6 mg/l (0.031 mg ± .018 mg per pad). This last value represents the maximum variation that can be expected. However, since in any one test all the filter pads came from the same set, the variability can be assumed to be that found for the series of tests using 0.025 N $K_2Cr_2O_7$, or a standard deviation of 1 mg/l when using a sample size of 20 ml. Thus it can be seen that the minimum accurately measured particulate COD is in the order of 10 mg/l with a standard deviation of about ten per cent when a filter is included with the blank (to correct for the added COD) and the sample size is 20 ml. Since the sample filtered, in order to measure low values of COD,was usually greater than 50 ml it can be seen that the minimum accurately detectable COD value would be less than 10 mg/l.

Organic Nitrogen

Organic nitrogen was determined by the micro Kjeldahl method followed by steam distillation and direct nesslerization of the distillate. The micro Kjeldahl method used in this study was a combination Standard Methods (170) procedure and that outlined by Kirk (174). The digesting solution was modified as follows: 80 gm of potassium sulfate, and 1 gm of SeO_2 was added to 942 ml of deionized water in a 4 liter beaker. Then 10 gms of $HgSO_4$ was added in a solution of 12 ml concentrated H_2SO_4 diluted to 100 ml with water. After addition of these chemicals the beaker was then placed in a cold-water bath and 1000 ml of concentrated sulfuric acid was added slowly with mixing.

Five milliliters of this solution were added to a sample (between 5 and 20 ml) contained in a 30 ml capacity micro Kjedahl boiling flask and then digested. Each sample was digested for 30 minutes after the digesting solution turned clear. The mixture was then cooled and 2 to 5 ml of superoxol (20% H_2O_2) was added to oxidize any organic matter that remained after digestion and also to wash all the material that condensed on the neck of the flask back into solution. After 5 more minutes of digestion the flasks were removed and cooled in preparation for distillation.

The samples were transferred by three rinses of deionized H_2O to flasks fitted with ground glass joints. These flasks were part of a steam distillation apparatus as described by Markham (175). One drop of phenolphthalein was added to the cooled digestion mixture and enough 30% NaOH was added to neutralize the mixture to the pink phenolophthalein endpoint. The digested sample was then attached to the distillation apparatus which was connected to a steam generator. Steam was passed by a glass tube to the reaction vessel where the ammonia was driven out of the alkaline digested sample into a condenser and collected in 50 ml nessler tubes. Analysis indicated that most of the ammonia was distilled off in the first fraction of a minute (first several ml), but a distillation period of at least 2 minutes (or distillation of at least 10 ml) was used in order to ensure complete recovery. The distillate was then analyzed by direct nesslerization as in the ammonia test.

The error involved in the digestion and distillation procedure was checked by digesting and distilling a series of concentrations of ammonium salts ranging from 0.010 to 0.110 mg N (equivalent to 1 to 11 mg/l N using 10 ml samples). Recovery of ammonia ranged between 95 and 106 per cent. In another test of ten duplicate samples the standard deviation of recovery was found to be 3.4 per cent of the average (0.103 mg/l - nitrogen).

Standard Methods (170) indicates that for the range of 1 to 5 mg/l organic nitrogen using regular 500 ml Kjeldahl flasks, 97.5 to 98.6 per cent was recovered with a precision of 0.03 mg/l. Considering the scale down in size of sample and equipment and ease of manipulation, the micro method was considered more appropriate, especially in cases where the sample size was limited to 10 to 20 ml as in this study.

Organic Nitrogen Content of Suspended Solids

In order to obtain a direct measure of the nitrogen of the particulate matter (seston as defined by Ruttner (176)) the suspended solids on the glass filter pad (as described in the suspended solids test) were digested as in the micro Kjedahl test described above. A factor which introduced a variable error in this test was the form of the nitrogen in the algal cell. A separate test was conducted to determine if the nitrogen in the suspended solids on the glass pads could be removed by several washings with deionized water and drying of the algae at elevated temperatures. It was found that the organic nitrogen in the particulate matter on undried glass filter pads was approximately the same as that found by subtracting the ammonia of the supernatant liquor (cells allowed to settle naturally) from the total micro Kjeldahl nitrogen. However, washing of the cells with a volume of deionized water equal to ten times the sample volume decreased the micro Kjeldahl nitrogen values an average of 10 per cent (varying from 0 to 17 per cent at a total organic nitrogen concentration of 5 mg/l) with a large variation depending on the algal genera and algal age. It was assumed that the observed nitrogen decrease was caused by a decrease in the cellular nitrogen rather than washing off nitrogen contained in the water remaining on the filter pad, since the soluble Kjeldahl nitrogen was less than 0.8 mg/l nitrogen in all cases. Drying of the sample for periods of up to 24 hours at 110°C caused a variation of the measured nitrogen (from that of unwashed undried cells of 0 to 32 per cent. Thus, in order to keep the loss of nitrogen from the glass pads at a minimum, no washing and a minimum of drying time (one hour) was used with the pads to be analyzed for particulate organic nitrogen.

The digestion of the filter pad in the determination of the particulate nitrogen added another error to the Kjeldahl test since the filter pads had an organic nitrogen content which varied from pad to pad.

For ten pads the average Kjeldahl nitrogen content was found to be 0.014 mg nitrogen with a standard deviation of 0.0011 mg nitrogen or 7.9 per cent of the average. Considering the precautions taken and the recovery of nitrogen obtained with the distillation procedure outlined, it is felt that the particulate nitrogen could be determined at a concentration of 0.10 mg/l using a sample size of 100 ml with a standard deviation around ten per cent. This value is below the minimum particulate nitrogen concentrations reported in this study.

Ammonia Nitrogen Determination

Ammonia nitrogen was determined either by direct nesslerization as given in Standard Methods (170) or by difference in cases where direct nesslerization was not possible. A number of different ions cause interference in the form of turbidity in the direct nesslerization process. In these cases ammonia was estimated as the difference between the total and particulate Kjeldahl nitrogen. The only samples where direct nesslerization was consistently non-applicable was with the sea water and estuary samples. Some amines, organic chloramines, acetone, aldehydes and alcohols, as well as other undefined compounds have also been found to yield a yellowish or greenish off-color upon direct nesslerization (170). No significant interferences such as these were observed.

The greatest source of variable error in the ammonia nitrogen test came from absorption of NH_3 from the air. The color concentration of nesslerized samples did not vary significantly after full color development for periods as long as 24 hours. However, when ammonia was present in the air the absorption proceeded at such a high rate as to cause a noticeable change in the blank reading over a period of seconds. Precautions were taken to always cover samples to prevent such absorption at all times. Whenever possible, no test was made while anyone was working with volatile ammonia compounds.

Digestion of organic nitrogen also had to be suspended when ammonia was present in the air.

The accuracy of this ammonia test depends mainly upon the presence or absence of turbidity. Since no turbid ammonia values were recorded in this study, the precision reported by Standard Methods of 5% should be the precision involved in this test. Caution is noted by Standard Methods (170) as seventeen independent laboratories came only within ± 23% of the known ammonia-N value in river water at a level of 0.40 mg/l.

Nitrite Nitrogen Determination

Nitrite nitrogen was determined by the alphanapthlyamine sulfanilic acid method described in Standard Methods (170). A number of ions are noted as interfering with this test due to precipitation. Included in this group are ferric, mercurous, silver, lead, and others. No precipitation was observed with any of the algal samples in this test.

Nitrate-Nitrogen

A number of different variations of nitrate nitrogen analysis were investigated for use with this study. All methods were modifications of the Brucine method (170). Jenkins and Medsker (177) modified this test so that it could be used with a minimum of error in both fresh and salt water, and were able to obtain a coefficient of variation of 1.7% in a series of ten replicate samples, each containing 0.03 mg/l nitrate-nitrogen.

In a similar study by these workers using the unmodified Brucine Method (170) a coefficient of variation of 17.7% was obtained.

Throughout the major part of this study Jenkins and Medsker (177) modified Brucine method for nitrates was used. Strict measures were taken in this test to adhere to the proper timing (to the second) and the temperature of each step in the test. Although this method was less susceptible to chloride interferences, there was still a radical variation between separate analyses. The only way this test was found to yield usable results in the low range of nitrate concentrations (0.5 mg/l NO_3-N) was when several standards were included with each analysis so that an appropriate standard curve could be drawn. Near the end of the testing in this investigation a very simple modification of Jenkins and Medsker's nitrate method was used and it eliminated most of the variability from test to test (178). This modified test was used in Experiment V only.

Using Jenkins and Medsker's nitrate method (177) twenty duplicate samples were analyzed on the same algal filtrate which had a nitrate nitrogen level of approximately 2.2 mg/l. The standard deviation found was 3.8 per cent of the average. The maximum variability of this test over a long period of time was measured by comparing the variation from the average color development of the standard nitrate concentration run at 0.5 mg/l-nitrogen. For 79 completely separate tests the standard deviation of the standard nitrate concentration was 12.4 per cent.

Nitrogen Balances

Complete nitrogen balances in biological systems are important in a complete definition of the nitrogen cycle. Although an accurate nitrogen balance at all points would have been interesting in this study, this was not necessary to fulfull the objectives. However, an attempt was made to account for all major nitrogen fractions throughout the study. The total nitrogen content of nearly all systems was adjusted to 5.0 mg N/l. For 87 separate samples of natural decaying algae (at all stages of growth) the average sum of the particulate nitrogen and ammonia, nitrite, and nitrate in the filtrate was 4.95 mg/l nitrogen with a standard deviation of 0.74 mg/l (or 14.8 per cent of the average). Since the organic matter in solution usually contained less than 10 mg/l organic carbon, it was assumed that the soluble organic nitrogen content was low and its exclusion would not introduce a significant error. This was checked in several instances and found to be true.

The error involved in the nitrogen balance can be attributed to many factors with the largest variable being introduced by: 1) absorption of ammonia from the atmosphere, 2) extraction of ammonia from the filtrate, and 3) variability of the nitrate test.

Phosphorus

Total and ortho phosphorus were measured using the following procedures on both unfiltered samples and on the filtrate using reagents as described in Standard Methods (170). A deionized water blank and standard were always carried through the procedure:

1. Place appropriate sized sample in a vycor dish.
2. Add 1 ml $MgCl_2$ (100 gm/l), and evaporate on steam bath.
3. Place in 103°C oven to dry and then burn at 600°C.
4. Cool and add 20-30 ml of water.
5. Add 1 drop phenolphthalein indicator. If sample turns pink add strong acid solution drop by drop to discharge color.
6. Proceed using procedure for total hydrolyzable phosphorus which is given below.
7. Add 1 ml of strong acid to the sample.
8. Heat in autoclave for 30 minutes at 15-20 psi.
9. Cool, neutralize to a faint pink with 1 N sodium hydroxide. Do this step to the closest half drop of NaOH. From this point on the procedure is similar for ortho phosphorus.
10. Pour into nessler tube and dilute to 50 ml, and mix with clean stopper over the tube end.
11. Add 2 ml molybdate solution and mix.
12. Add 5 drops stannous chloride solution and mix.
13. Wait 10 minutes and read % transmission at 690mu, but read all samples before 12 minutes.

Lee et al. (179) investigated a number of different phosphorus analyses with the object of determining their applicability to phosphorus determinations in the presence of algae. The total concentration of phosphorus used was the same as that used in nearly all of this work--0.5 mg/l phosphorus. They found that the ashing test for total phosphorus, similar to that described above, was more accurate than other methods, although the time-consuming manipulation of the sample was considered detriment over the other methods.

Ortho phosphorus was determined on the filtrate of algal systems. This procedure is outlined in steps 9-13 in the total phosphorus procedure outlined above.

The total phosphorus analysis is a good test and is susceptible to few interferences. However because of its sensitivity (50% transmission approximately equal to 0.01 mg P) and the many steps involved, an appreciable testing error may result.

Analysis of the results from this study show that there may be two major sources of error: 1) variability of factors within one test, and 2) variability over a period of several tests. Twenty duplicate samples of the same filtrate from an algal system showed that the ortho phosphorus procedure had a standard deviation of 5.8 per cent at an average ortho phosphorus concentration of 0.25 mg/l phosphorus. This should represent the minimum error. However, analysis of the variability of the phosphorus standard (1.0 mg/l P using a 10 ml sample) that was run with each test showed a standard deviation of 3.82 per cent of the average for the ortho phosphorus analysis and 5.67 per cent for the total phosphorus analysis.

Standard Methods (170) states that the minimum detectable concentration for the ortho phosphorus test is 0.003 mg/l phosphorus using the stannous chloride method. Considering the number of steps in the total phosphorus test and the standard deviation observed with the algal samples, the minimum accurately detectable concentration of total phosphorus using the procedure outlined earlier is probably in the order of 0.01 mg/l phosphorus. All results reported in this study were higher than this value.

Dissolved Oxygen

Dissolved oxygen was determined by the alsterberg (Azide) Modification of the Winkler Method, as indicated in Standard Methods (170).

Rate of Oxygen Uptake Determinations

The rate of oxygen consumption was measured by one of the following two methods depending on the required precision and nature of the sample:

1. Biochemical Oxygen Demand Test (BOD).
2. Warburg Anaylsis

The biochemical oxygen demand test used was similar to the Standard Methods procedure except for the composition of the dilution water. Since the objective of this test was to approximate the biological action that was taking place in each experiment, it was felt that addition of an outside source of nutrients would not simulate the actual system. Thus deionized water was used for dilution of most samples. Because of the relatively low oxygen uptake rates and the resulting low dilution factor (usually less than 6:1) the error introduced by using an unbuffered nonsupplimented dilution water was minimal. Numerous BOD_5 tests using duplicate samples--one with deionized water and the other with the standard dilution water (except for the substitution of nitrate for ammonia) showed this to be true for all types and ages of algae.

In appropriate experiments, bacterial and animal seed was added to each test. However, in BOD determinations of pure cultures of algae, the bottles were sterilized before use.

The standard deviation reported by Standard Methods (170) of 0.07 to 0.11 mg oxygen demand should hold for this test.

The Warburg method (using a Precision Warburg Apparatus manufactured by Precision Scientific Company) for determining oxygen uptake was also used sparingly. The Warburg method used was listed as a tentative procedure in Standard Methods 11th Ed. (1960).

Organic Carbon

All carbon analyses were conducted using a Beckman Carbonaceous Analyzer. The analysis system consists essentially of a panel on which are mounted a Beckman Model 1R-315 infrared Analyzer, a Leeds and Northrup type "H" Model "S" Strip-chart recorder, and a special sampling system. The sampling system consists of an injection port where the sample is introduced into the analyzer using a 50 micro-liter syringe. The sample is then carried into a combustion tube (held at 950°C) by a flow of pure oxygen (50 ml oxygen per minute). All the organic matter is burned in the combustion tube and the oxygen flow then carries the combustion products into the infrared analyzer where carbon dioxide causes a differential absorption of the infrared light. This is quantitatively measured and the output is shown as a sharp curve on the recorder chart.

The range of the test for organic carbon may be adjusted depending on the concentration that is expected in the sample. Two different scales were: 1) full scale equal to 100 mg/l carbon, and 2) full scale equal to 50 mg/l carbon. The adjustment of the range of the instrument was accomplished by standardization of the full scale deflection of the instrument with the desired concentration of carbon. After this point was determined, the standardization curves were obtained using a series of dilutions of a carbon standard which was prepared from ACS grade glacial acetic acid (preserved over long periods of time with the addition of $HgSO_4$.

Sample Preparation for Total Organic Carbon Test

Because inorganic carbon in water samples is measured by the carbon analyses, pretreatment of the samples was neeessary. In all cases the samples were acidified and aerated with CO_2 free nitrogen for a period of not less than 5 minutes. Acidification was obtained in each case by the addition of 1 drop of concentrated HCl to 10 ml of sample.

To determine the effect of acidification and nitrogen aeration on volatile organic matter and the speed with which CO_2 is driven off, the following test was performed, using $NaHCO_3$ at a level of 50 mg/l C and acetic acid at 50 mg/l C. A series of samples were acidified as noted above and aerated with nitrogen for varying periods of time. The results are shown in Figure 11 and indicate an extremely rapid loss of the alkalinity carbon but little loss of the volatile organic carbon. Further aeration of this mixture for 30 minutes caused no reduction in the organic carbon. These results would not hold for organic material such as acetone, but volatile organics of this type were not considered to be significant in this study.

Filtrate Organic Carbon

To determine the amount of soluble organic carbon a portion of the filtrate of each sample was acidified and aerated with nitrogen as described above for a minimum of 7 minutes. Since the error in the carbon test for soluble organics will approach the variability introduced by testing equipment error only, the filtrate organic carbon was considered to be more reliable than the total organic carbon. For this reason the chemical oxygen demand of the filtrate was seldom run.

The error in the testing method with soluble organics such as the

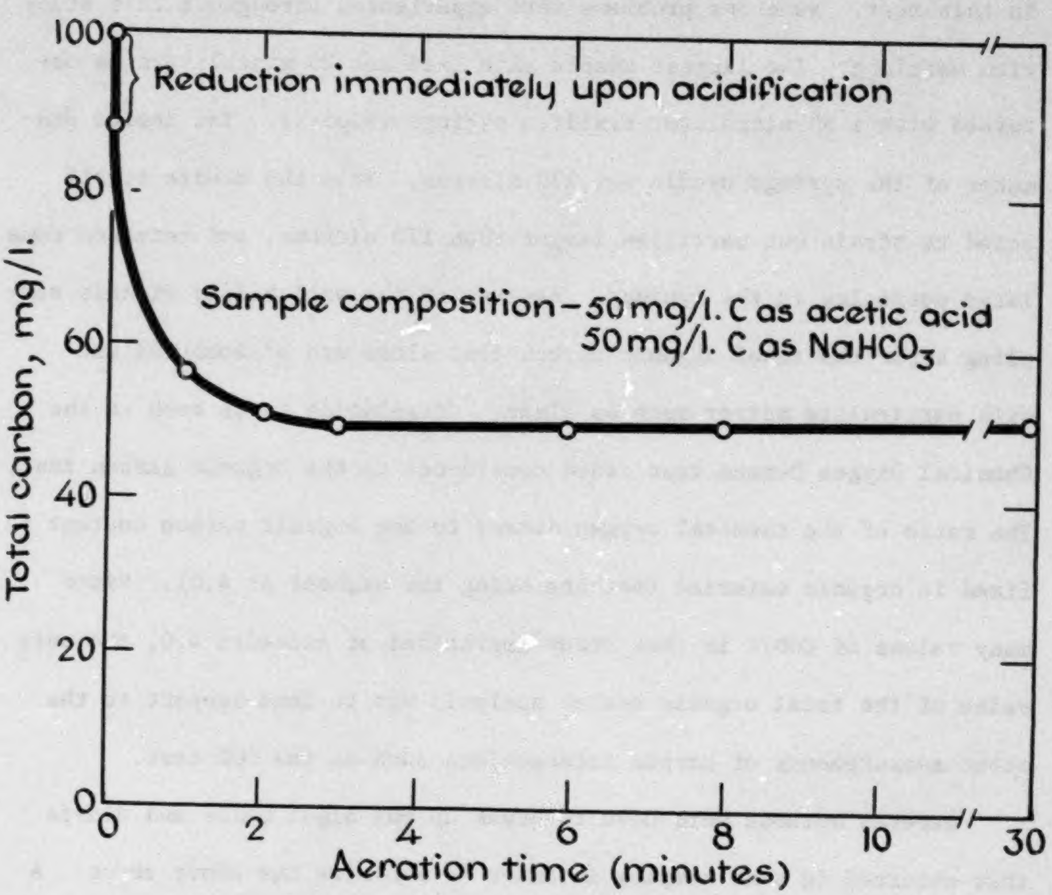

FIGURE 11. Rate of inorganic carbon removal from an acidified sample prior to organic carbon analysis.

acetic acid standard was less than 1 mg/l C in the range of 10 to 100 mg/l carbon. However, the sampling error was the largest source of error in this test. Numerous problems were experienced throughout this study with sampling. The largest sample size used was 20 microliters as obtained with a 50 microliter Hamilton syringe (#705-N). The inside diameter of the syringe needle was 170 microns. Thus the needle itself acted to strain out particles larger than 170 microns, and retained some large particles in the syringe. Because of the variability of this sampling error the total organic carbon test alone was of doubtful use with particulate matter such as algae. Correlating tests such as the Chemical Oxygen Demand test added confidence to the organic carbon test. The ratio of the chemical oxygen demand to the organic carbon content is fixed in organic material (methane being the highest at 4.0). Since many values of COD/C in this study approached or exceeded 4.0, the only value of the total organic carbon analysis was to lend support to the other measurements of carbon interactions such as the COD test.

Several methods were used to break up the algal cells and clumps that occurred in some samples in order to minimize the above error. A relatively gentle blending (Servall-Omnimixer, 16,000 RPM) of some samples was adequate. In most cases it was necessary to shatter the cells with an ultrasonic vibrator (Heat Systems Company, Ultrasonic Devices, Great Neck, New York).

Lipids

Total lipids or total chloroform extractables include fats, oils, waxes, and other organics soluble in chloroform. Whenever the term lipid is used in this study it will refer to total chloroform extractable material since further purification was not performed. The method used was a modification of the procedure outlined by Loehr (187):

1. Acdify to pH 3 a large enough sample to obtain approximately 50 mg of extractables.
2. Transfer acidified sample to blender. Add 40 ml chloroform and 80 ml methane to blender (measured in order stated).
3. Mix sample and solvents for 1 minute.
4. Add additional 40 ml chloroform and mix for 30 seconds.
5. Add 50 ml distilled water and blend again for 30 seconds.
6. Filter through Buchner funnel.
7. Transfer flask contents to separatory funnel and wash flask with 25 ml chloroform and 25 ml methanol. Add washing to separatory funnel.
8. Shake separatory funnel for 1 minute and allow to stand until phase separation is complete.
9. Draw off chloroform layer into previously tared beaker. Evaporate chloroform on steam bath. Cover beaker with filter paper to prevent absorption of dust. Purge fumes from beaker with air and complete drying in dessicators.
10. After approximately 24 hours weigh the tared beaker.

Chlorophyll

Chlorophyll was determined using the approach based on MacKinney's work (181) and further developed by Arnon (182). Several investigators (183, 184, 185). indicated that direct quantitative extraction of chlorophyll with 80% acetone and water was possible with unicellular algae.

This method was used for a large portion of this study until it was found to be invalid for quantitative determination of chlorophyll in older algal systems. The following procedure was found to be adequate under all conditions and is the method by which all results reported were made:

1. Filter enough algal material onto a glass filter pad as described in the suspended solids test. The size of the sample needed depends on the chlorophyll concentration.
2. Add $NaHCO_3$ for a buffer against the release of acid material upon crushing of the algal cell. Immediately after filtration, grind the filter pad and the algae in a mortar and pestal vigorously for two minutes.
3. Add 10 ml of 80% acetone and H_2O and continue grinding for 1 minute.
4. Filter the resulting mixture as in suspended solids determination. The suction apparatus should be modified so that the acetone filtrate can be caught in a test tube inside the vacuum flask.
5. Read the absorbance of each sample using 1 cm path length (in Beckman DB spectrophotometer).
6. Apply the following equation as originally given by MacKinney (175):

$$G = 20.2\ D_{645} + 8.02\ D_{663}\ F$$

where G is the total chlorophyll concentration (chlorophyll a plus b) mg/l, D is absorbance units @ 645 A° and 663 A° wave length, and F is the dilution factor = ml 80% Acetone/ml sample.

Sources of Error

There are two sources of error that must be considered in this study. They are: 1) testing errors, and 2) errors in the postulated hypotheses. Testing errors include the inherent variability and accuracy of the test itself and human error. The testing errors have been summarized earlier in this section under each individual test. It was shown, for example, that the COD test had a standard deviation of about 10% of the mean. This indicates that the measured value for the COD is usually close to the actual value. However, not included in any of these test errors is the variability introduced by the changing characteristics of the algae with time. During the growth of algae there were stages where a substantial fraction of the algae grew in clumps or stuck to the walls. Special precautions were taken to keep significant attached growth from forming so that representative samples could always be taken. Triplicate and quadruplicate samples were used in many instances. However, it can be seen that when dealing with 50 mg/l total COD or 0.25 mg/l P that a large particle of organic matter could easily change the results by more than ± 50% of the actual value. This is not to say that the testing was always in error by 50%, since most tests used in this study were quite precise and were applied with utmost caution. This does point out that obtaining a representative sample from an algal system is perhaps the most difficult analytical problem in this study. In many instances a compromise between the desire to homogenize the algal system and the desire to keep the algal systems in as natural a state as possible had to be made. This usually resulted in working with a few large clumps of organic matter in the sample. Duplicate and triplicate samples were taken at most important points. However, algal species such as in the latter stages of the "Synthetic River Water" (No. 6) sample remained a problem. This algae was a blue-green Anabaena type that grew in very tightly packed chains. Ultrasonic vibration used effectively to completely disintegrate most bacteria and algae in 60 seconds failed to unravel these chains. However, in the large majority of the samples vigorous shaking was all that was necessary to obtain adequate dispersal of the solid organic matter. In several cases it was necessary to transfer the whole sample to a Waring Blender and blend at high speeds for 15 to 20 seconds. This treatment did not appear to affect the algal cells since no parameter such as dissolved organic carbon or phosphorus appeared to change significantly. The majority of sampling was done by pipette from cultures being mixed rapidly with a magnetic stirrer.

A good example of the reproducibility obtained with the combination of the aerating system, the sampling technique, and the COD test is the results obtained with the young natural algal cultures. These cultures were placed in the dark at a total COD of around 40 mg/l. After several weeks the total COD decreased to 20 to 30 mg/l. From this point on for a year and a half the measured COD did not change more than several mg/l. For example, in one sample the total COD varied only between 10 and 12.5 mg/l over a period of 9 months.

Another major source of testing variability stems from the ecological approach taken in this study. In order to simulate natural conditions in the laboratory, no attempt was made to control the algae, bacteria, and animal populations that grew in the samples. As a result the populations in all natural samples were vastly different. Whether any general conclusions at all could be drawn from such a study dealing with many different types of water and algae was questionable at the onset. Thus it should not be surprising that significant differences occurred between samples and in the same sample from one time to another.

V. EXPERIMENTAL RESULTS

The following experimental results summarize numerous algal growth and decomposition studies ranging in length from several hours to several years. These results are separated by experiment with a description of the experiment and the general objective given in each case.

EXPERIMENT I

High Nutrient Level Algal Growth and Decay

In the first series of experiments the major objective was to determine whether algal growth and decay could be simulated in the laboratory, and if so, whether it posed as great a potential problem as would be expected based on comments made in the literature. Three different nutrient solutions were used in this experiment as shown in Table 11.

Table 11. Algal Growth Media Used in Experiment I.

	NAME OF MEDIA		
CHEMICAL	UREA (Conc. per liter)	NH_4Cl	ACTIVATED SLUDGE EFFLUENT
$MgSO_4.H_2O$	5.0 gm	Same as urea except using NH_4Cl instead of urea as a nitrogen source.	Activated Sludge effluent from Stanford University pilot plant at Palo Alto wastewater treatment plant. Approx. 25 mg/l N, 8.5 mg/l P.
KH_2PO_4	2.5 gm		
NaCl	2.0 gm		
Nitrogen	37.3		
Na_2EDTA	4.0 mg		
$CaCl_2$	22.0 mg		
H_3BO_3	5.7 mg		
$MnCl_2 \cdot 4H_2O$	3.6 mg		
$ZnSO_4$	0.44mg		
$CuSO_4.5H_2O$	0.16mg		
$(NH_4)Mo_7O_{24}.5H_2O$	0.035 mg		

The nitrogen and phosphorus concentrations ranged from 25 to 37.3 mg/l N and 8.5 to 579 mg/l P. The algae were grown in the growth apparatus described in the Experimental Procedures Section. The algal seed was obtained from a eutrophic lake (Searsville Lake). After growth occurred, samples were taken at various algal ages and placed in total darkness and aerated with the air-CO_2 mixture described previously. All of these samples were taken while the algal cultures were in an exponential growth phase. A comparison of autoclaved dead cells versus live cell decomposition was made with samples from NH_4Cl and Activated Sludge effluent media.

Variables measured in this series of tests were as follows: chemical oxygen demand (COD), total organic carbon and biochemical oxygen demand (BOD). The results from this series of experiments were used as guides for the design of future experiments. Table 12 gives a summary of some of the pertinent information obtained. Although there was a good deal of variation in the data, several factores were significant. Most important, in about seventy-five per cent of the samples (13 out of 17, not including autoclaved samples) after decomposition periods ranging between 10 and 345 days, the remaining refractory material equaled greater than 40 per cent of the initial COD, and in nearly fifty per cent of the samples the refractory material represented greater than fifty per cent of the initial material.

The percentage of refractory organic material in the different samples indicated two significant factors. First, there appeared to be an increase as the algal system aged, thus indicating added resistance to decomposition due to age, and secondly, in two cases the first

Table 12. Summary of the results of the decomposition of algae grown in media with relatively high nutrient concentrations (Experiment I).

SAMPLE	ALGAL AGE (days)	DECOMP. PERIOD (days)	INITIAL COD (mg/l)	FINAL COD (mg/l)	PER CENT COD REMAINING
NH_4Cl	5	10	77.5	46.5	55.4
	8	121	99	135	136
	17	336	300	161	53.7
	23	106	430	236	55.0
	31	320	636	348	53.2
	46	83	1500	896	59.7
	46*	83	1500	539	35.9
	17	304	704	313	44.4
	17*	304	720	288	40.4
Urea	6	345	165	142	86.0
	19	334	257	105	40.8
	23	330	315	97	30.8
	31	320	458	155	33.8
	46	83	522	338	64.7
Activated Sludge Effluent	6	121	23.8	15.3	64.2
	17	334	194	32.1	16.4
	23	334	318	149	46.8
	31	330	404	166	41.1
	48	320	702	254	36.1
	48*	307	516	106	20.5

*Killed (autoclaved)

young algal samples had a refractory organic fraction higher than the older algae from the same media. The 8 day old culture in the NH_4Cl media had an increase in COD over a period of 121 days of decomposition. This was assumed to result from synthesis of autotrophic nitrifying bacteria and may have occurred in other samples as well. In the three autoclaved samples the decomposition proceeded faster and to a greater extent than with the live controls.

The results of this experiment indicated that algal material was unique in that it possessed a large fraction of material that was resistant to biological decomposition. Since few researchers in the past have noted this and no known study has been done to explain this phenomenon, it was felt that further study was justified. However, the high nitrogen and phosphorus concentration in the growth media (Table 11) did not represent a concentration which might be found under most circumstances in natural bodies of water. It was also felt that these high nutrient concentrations would not stimulate normal algal growth since it has been shown that 20 mg/l of nitrogen and/or phosphorus causes growth inhibition of some species (144). Thus in all future experiments the initial nitrogen and phosphorus concentrations were always less than 5 and 0.5 mg/l, respectively.

EXPERIMENT II

Treatment Plant Effluent Grown Algae

Algal growth experiments on various waste treatment process effluents were being conducted in the laboratory (189) during the initial stages of this study. These growth experiments were designed to demonstrate the growth potential of algae in both secondary and tertiary waste water effluents. The effluent samples were taken from the Stanford Water Reclamation Demonstration Plant's activated sludge unit located at the Palo Alto Wastewater Treatment Plant. Some of the activated sludge effluent samples were used directly, while others were treated using an anionic exchange resin to remove some of the nitrates and phosphates and then used as a growth media. The nitrogen and phosphorus concentration in the growth media ranged from .2 to 33 mg/1 N and a trace to 20 mg/1 P. The seed used in this series of tests had been obtained from a eutrophic lake (Searsville Lake).

The treated effluents had been placed in three liter Erlenmeyer pyrex flasks and exposed to continuous illumination provided by fluorescent lights (approximately 1000 foot-candles as measured 3 inches below the bulbs). Separate air and CO_2 aeration lines attached to aeration stones were used to continuously mix the samples. These growth studies were all terminated after a period of growth of 14 to 16 days. The decomposition of the algal cultures produced in this manner were then studied to give supporting evidence as to the biodegradability of algae.

At the end of the growth period 900 ml of the samples were placed in 1 liter vessels and placed in the dark to decompose under conditions similar to all other experiments. Periodic COD measurements of the decaying algae were made over a period of 253 to 330 days. The initial and final COD are shown in Table 13. The majority of the samples had a refractory organic fraction greater than 40 per cent with a maximum variation from 26 to 78 per cent. Thus this experiment provided further evidence that frequently less than 60 per cent of the algal cell was available for biological decomposition, and justified the need for further research.

Table 13. Results from Aerobic Decomposition of Algae Grown in Wastewater Treatment Plant Effluent.

MEDIA	DECOMPOSITION PERIOD (days)	INITIAL COD (mg/l)	FINAL COD (mg/l)	REFRACTORY FRACTION (%)
1. Ion Exchange Effluent	330	1720	780	45
2. " " "	330	1120	840	75
3. " " "	330	5660	4320	76
4. Act. Sludge Eff. plus Ion Exchange Effluent	330	3750	2000	53
5. " " "	330	3180	1456	46
6. Ion Exchange Effluent	330	1380	369	27
7. " " "	330	1110	342	32
8. Activated Sludge Eff.	330	4870	2720	51
9. Ion Exchange Effluent	330	1960	640	33
10. " " "	253	186	59	32
11. " " "	253	186	49	26
12. Activated Sludge Eff.	253	1432	751	52
13. " " "	253	1200	640	53
14. Ion Exchange Effluent	253	604	188	31
15. " " "	253	368	128	35
16. " " "	253	189	89	47
17. " " "	253	208	94	45
18. " " "	253	327	99	30
19. " " "	253	357	125	35
20. Activated Sludge Eff.	253	1738	1160	78
21. " " "	253	1690	1130	76

EXPERIMENT III

Major Experiments on Natural Water Samples

The experimental growth and decomposition apparatus had proven satisfactory in the initial experiments and the data obtained indicated that further research on the decomposition of algae was warranted. Thus an intensive study of algal growth and decay using a wide spectrum of natural waters was initiated. Water was obtained from twelve sources in California as shown in Table 14 with the first six samples being used in this experiment and the last six being used in Experiment IV.

Table 14. Natural Water Samples Studied.

IDENTIFICATION NUMBER	SAMPLE SOURCE	TYPE WATER
1.	San Pablo Bay (San Francisco Estuary)	Estuarine water
2.	Tidal Sacramento River (Carquinas Bridge)	Tidal river water
3.	Non-tidal Sacramento River	Slightly polluted fresh river water
4.	Searsville Lake (Stanford)	Eutrophic lake
5.	Activated sludge effluent (Stanford Univ. Pilot plant)	Treated effluent
6.	Synthetic River Water	See Table 16.
7.	Lake Tahoe	Oligotrophic lake
8.	Activated sludge effluent (similar to #5)	Treated effluent
9.	Folsom Reservoir	Man-made reservoir
10.	Rogers Creek (Big Basin Park)	Intermittent stream
11.	Clear Lake (Konocti Bay)	Eutrophic lake
12.	Clear unpolluted brook (Northern California)	Mountain stream

The natural water samples were filtered using glass fiber filters as described in the Procedure Section under suspended solids, and then aerated for several days to several weeks to oxidize most of the organic matter to carbon dioxide, and nitrogen forms to nitrate. While the aeration process was carried out, the debris removed in the filtration process was placed in 500 ml of the original sample water and was aerated slowly and exposed to dim diurnal light. After the samples had been aerated, it was found that the COD of the samples was less than 15 mg/l and the nitrate and ammonia-nitrogen concentrations were low or unmeasureable. In all waters used, the nitrogen and phosphorus concentrations were adjusted to approximately 5 and 0.5 mg/l respectively, by addition of nitrates and phosphates. Because the treated wastewater effluent (5) had an ortho P content of approximately 3.0 mg/l P, it was necessary to dilute this water six times with deionized water to achieve an ortho-P concentration of 0.5 mg/l P. Nitrates were then added to obtain a nitrate-N content of approximately 5 mg/l. Table 15 includes the initial composition of the first six natural waters shown in Table 14 and the adjusted nitrate and ortho phosphorus concentrations before algal growth was initiated. Table 16 indicates the composition of the "Synthetic River Water" (6).

The concentration of nitrogen and phosphorus of 5 and 0.5 mg/l respectively was used for several reasons. First, the experiments were designed so that they would give results applicable to the natural environment to the extent that "in vitro" experiments may simulate "in vivo" reactions. Thus nutrient levels close to what might occur in a natural situation were used. Secondly, the level of nutrients and the resulting growth should be sufficiently high to be in the accurate range

Table 15. Initial Composition of Natural Waters, and Final Adjusted Nitrate and Phosphate Concentrations.

INITIAL COMPOSITION

SAMPLE	TOTAL DISSOLVED SOLIDS (mg/1)	ALKALINITY (mg/1 as $CaCO_3$)	TOTAL HARDNESS (mg/1 as $CaCO_3$)	CHLORIDES (mg/1)	ORGANIC C (mg/1)	COD (mg/1)	AMMONIA-N (mg/1)	NITRITE-N (mg/1)	NITRATE -N		ORTHO P	
									INITIAL (mg/1)	FINAL (mg/1)	INITIAL (mg/1)	FINAL (mg/1)
1	17500	40	1400	6700	4.5	10.0	0	0.06	0.46	5.00	0.050	0.500
2	9600	40	850	4150	3.3	8.4	0	0.04	0.51	4.80	0.030	0.470
3	272	45	136	52	5.0	15.3	0.12	0	0.64	5.00	0.050	0.550
4	320	135	176	32	9.0	27.0	0.30	0.02	0.27	5.20	0.010	0.560
5	185	30	48	33	2.8	10.0	0	0.40	0.52	5.70	0.580	0.570
6	225	75	76	65	1.9	10.0	0	0	5.50	5.50	0.500	0.500

Table 16. Composition of Natural Synthetic River Water.

	STOCK SOLUTION		COMPOSITION OF RIVER WATER	
CHEMICAL	mg/l	ml added to make a liter of solution	ELEMENT	mg/l
$NaHCO_3$	1260	50	Na*	17.2
$FeCl_3 \cdot 6H_2O$	270	1	Fe	0.05
$CuSO_4 \cdot 5H_2O$	39.3	1	Cu	0.01
$MnSO_4 \cdot H_2O$	30.8	1	Mn	0.01
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	12.8	1	Mo	0.01
$NaVO_3$	23.9	1	V	0.01
KNO_3	3610	10	N	5.0
---	--	--	K	13.9
Na_2HPO_4	229	10	P	0.5
$MgSO_4$	496	100	Mg	10.0
$CaCl_2$	825	100	Ca	30.0
---	--	--	Cl	56.0
$ZnCl_2$	20.8	1	Zn	0.01
$CoCl_2$	2.2	1	Co	0.001

*Alkalinity 75 mg/l as $CaCO_3$

of available analytical tests. Thirdly, the level of nitrogen and phosphorus should be in an optimum range for growth of algae. Chu (144) and others (140) have determined inhibiting and optimum levels of nitrogen and phosphorus, and the level of nutrients chosen for this work falls within the optimum nutrient concentrations reported.

After pretreatment of the water samples by aeration, filtration, and addition of nitrogen and phosphorus, 18 liters of each sample was placed in the growth apparatus and reseeded with 2 ml of the debris that had been initially removed (contained in 500 ml untreated water) in an attempt to insure that the flora and fauna would be similar to the sample as collected. Within twelve days after the six samples had been exposed to diurnal light a bloom occurred in each unit.

In an effort to define the effects of age of the algal systems on the decomposition variables studies, an attempt was made to take six "branch" samples at different periods from each growth unit which would correspond to a given physiological condition of the algal populations. These different "branch" samples were to conform in general, to the following growth phases as denoted by the algal growth rate and nutrient conditions:

SAMPLE	ALGAL SYSTEM CONDITION
1.	Logarithmic growth, nutrients in solution.
2.	Logarithmic growth, nutrients just disappeared from solution.
3.	End logarithmic growth, nutrients gone from solution for long period of time.
4.	Declining growth, nutrients gone from solution for a long period of time.

5. Stationary phase, nutrient limitation causes cessation of growth.

6. Declining phase, nutrients start to come back into solution.

All the above desired samples, however, were not obtained except in two of the six natural waters because most of the cultures did not enter the stationary or declining growth stages for the entire length of the experiment. The 900 ml branch samples were taken at times ranging from eight to 211 days after the "bloom" appeared. The days after which "branch" samples were taken are shown in Figures 12 to 17. The samples were placed in total darkness at 20°C and aerated with a 1 per cent carbon dioxide in air mixture to evaluate the decomposition effects.

Growth in the six natural samples was studied over a period of 700 days, while decomposition of the branch samples was monitored for periods of up to a year and a half.

General microscopic examinations were conducted to determine the biological activity in all growth and decomposition vessels and to observe the predominant algal population. The identification of the algae was limited to generalized categories, i.e., diatoms, green algae, motile green, filamentous green, and blue-green. Animals were noted in general as being present in large or little mass and being very active or nonactive.

Growth of Algae

Figures 18A to 18F contain the summarized results of the growth in the laboratory of naturally occurring algae in the first six waters listed in Table 14. Included with the growth curves are the data for the total particulate organic nitrogen and phosphorus.

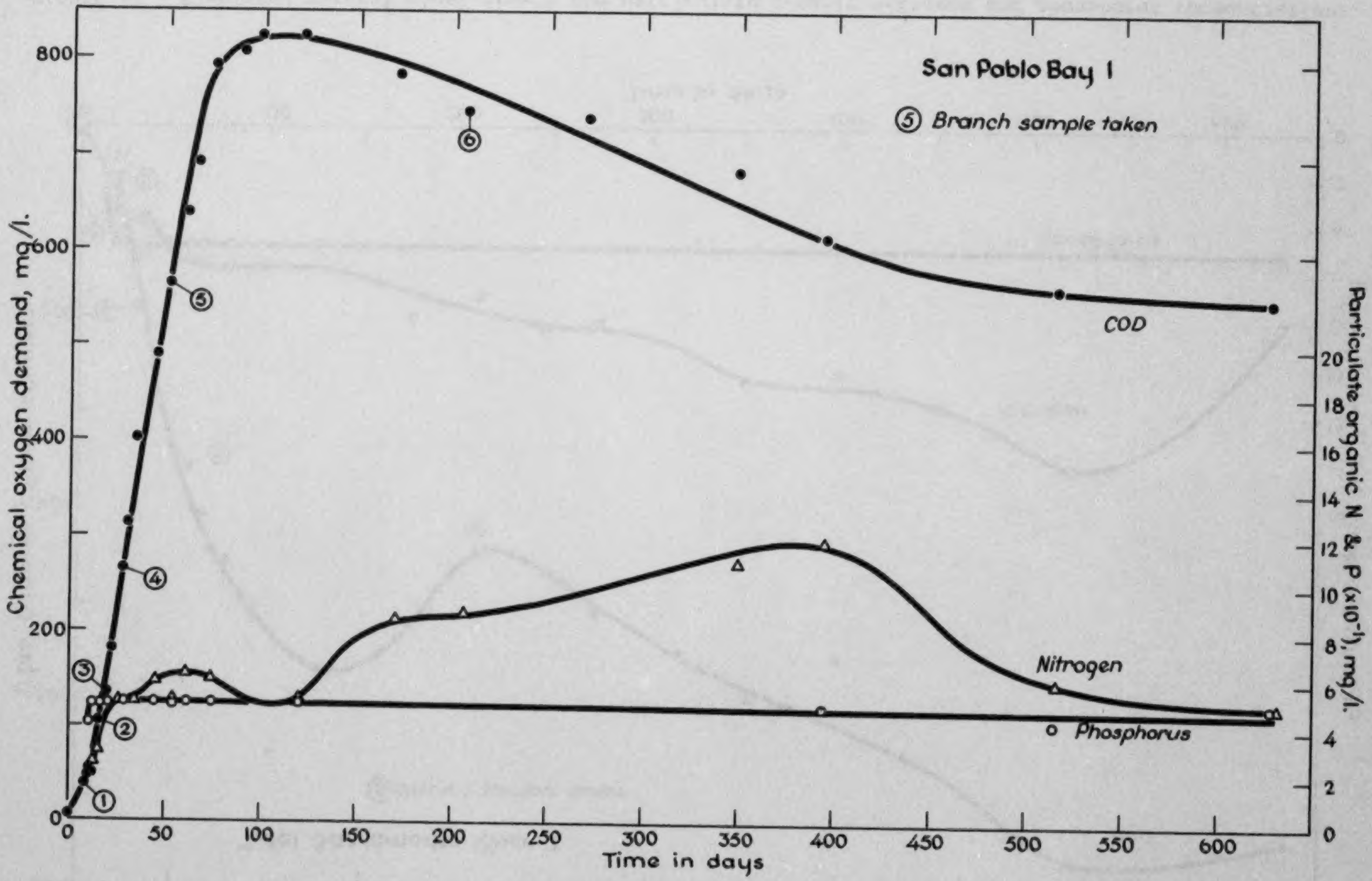

FIGURE 12. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations.

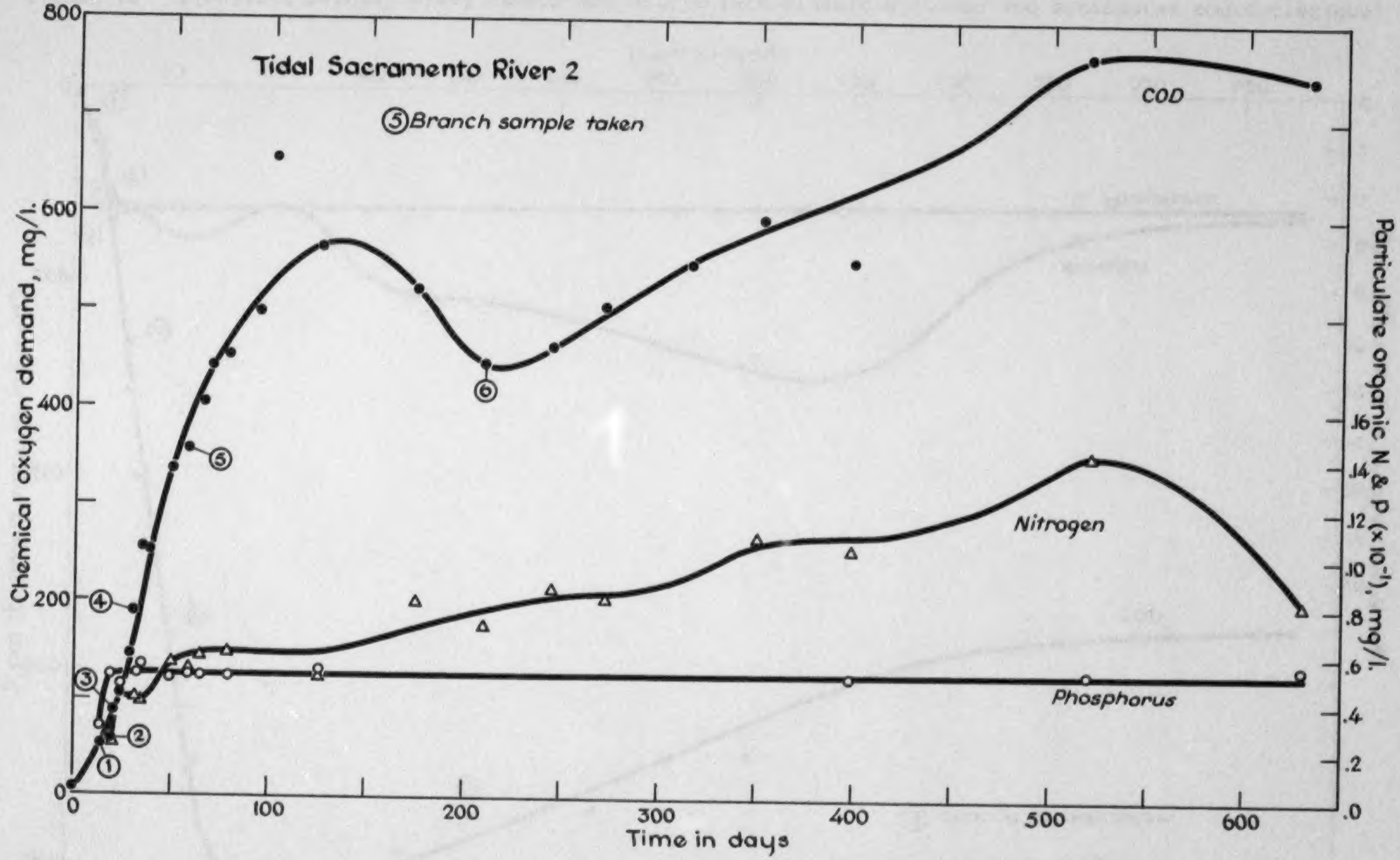

FIGURE 13. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations.

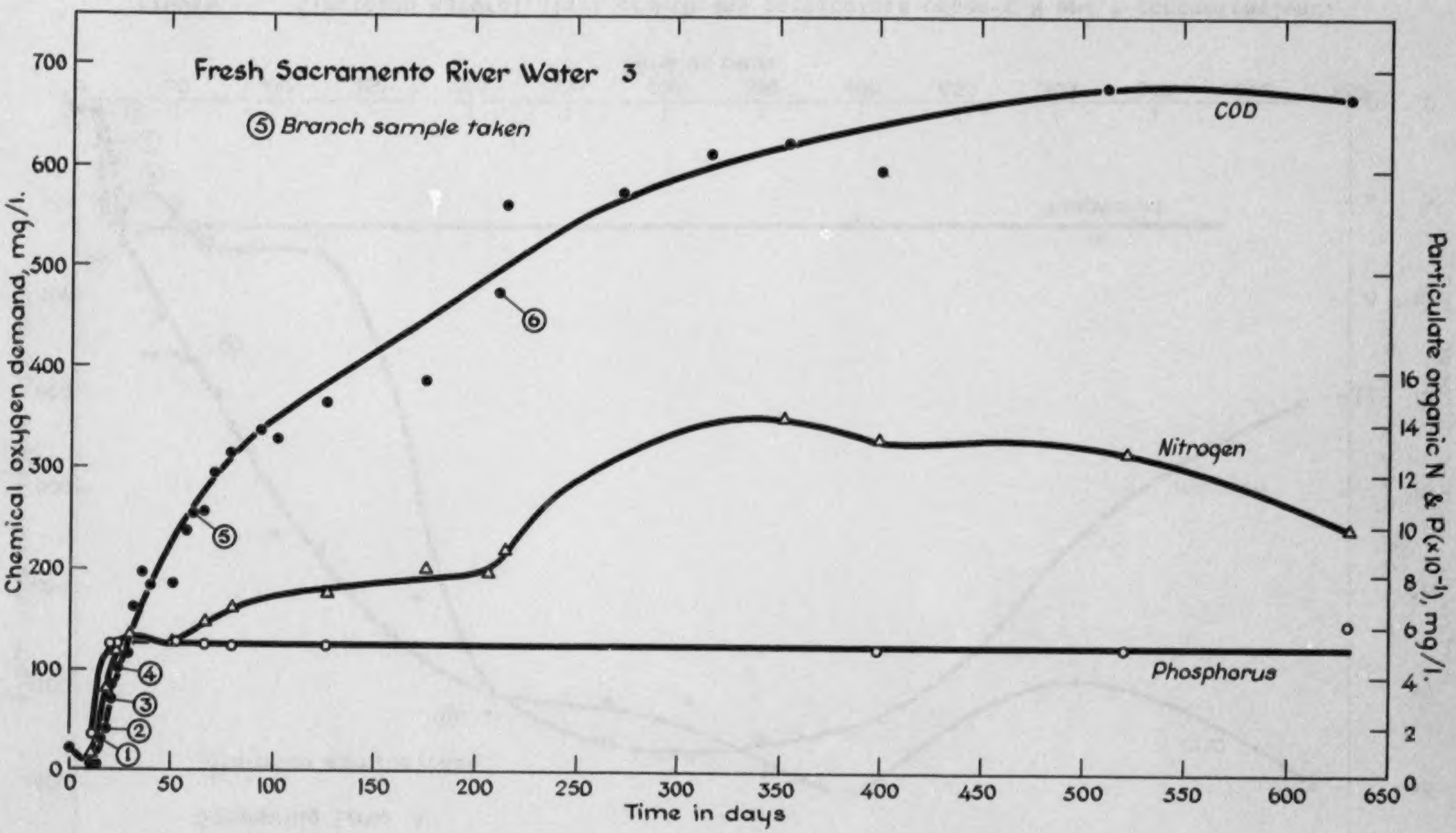

FIGURE 14. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations.

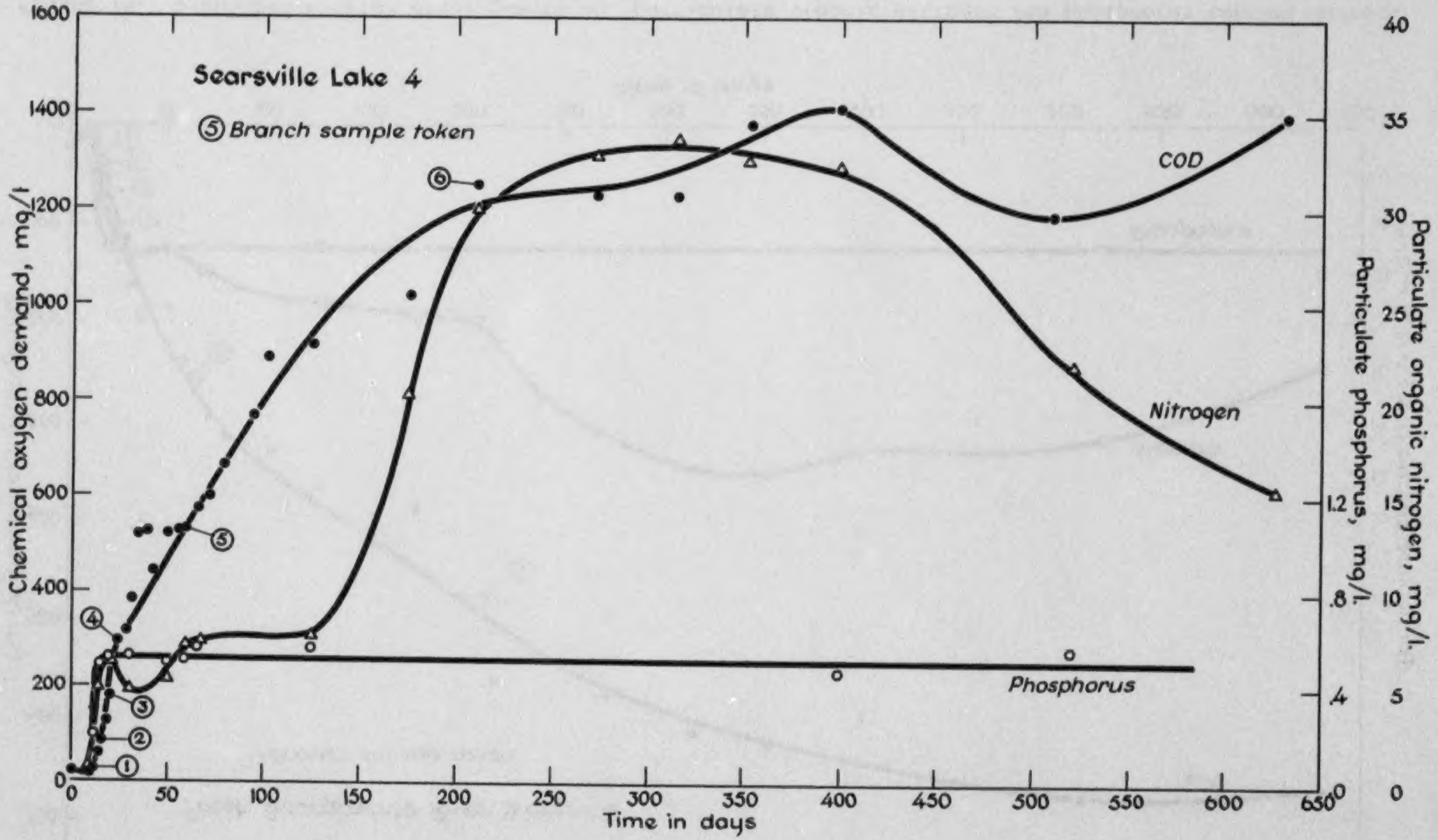

FIGURE 15. Simulated natural algal growth and particulate organic N and P concentrations.

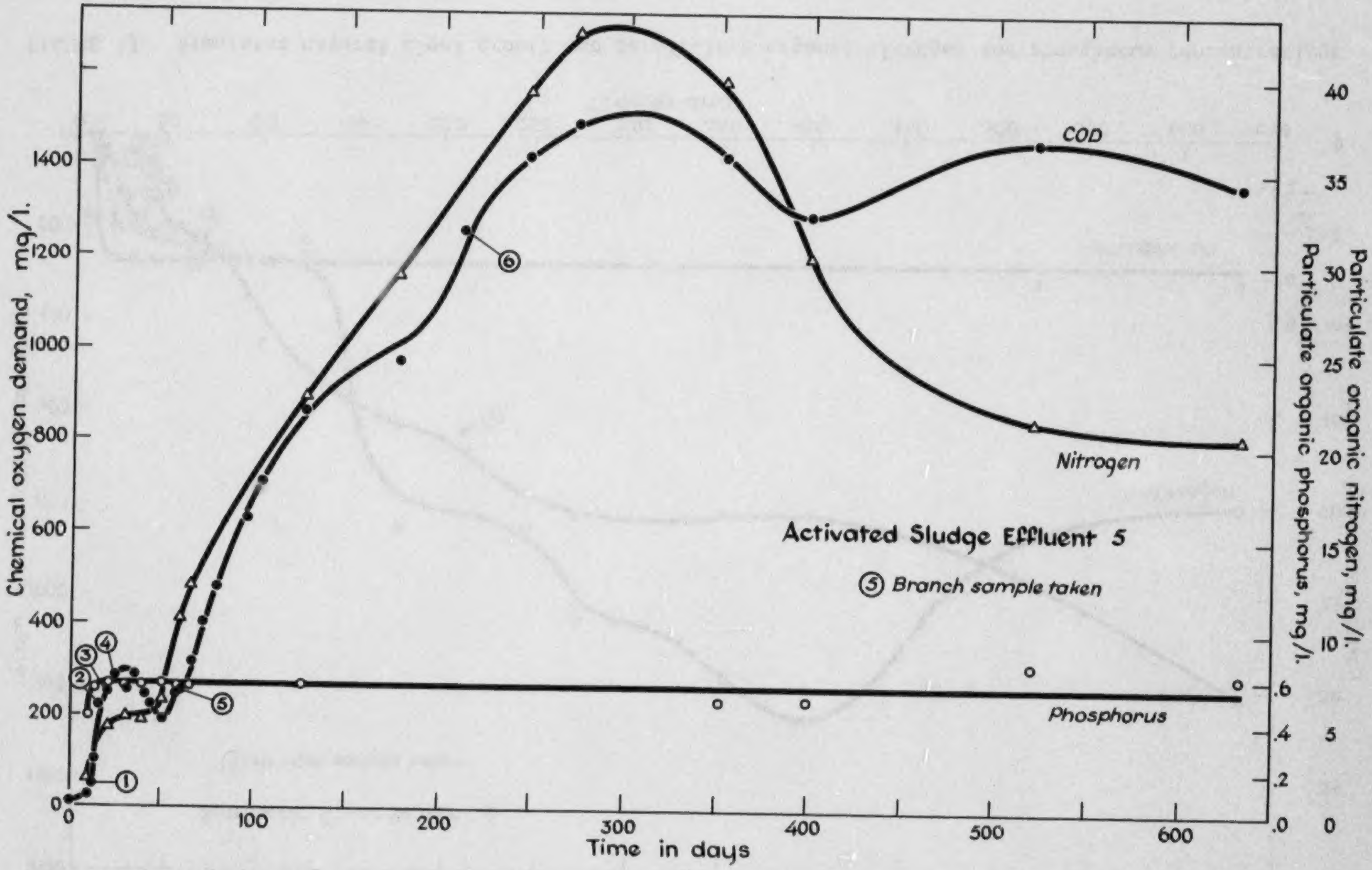

FIGURE 16. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations.

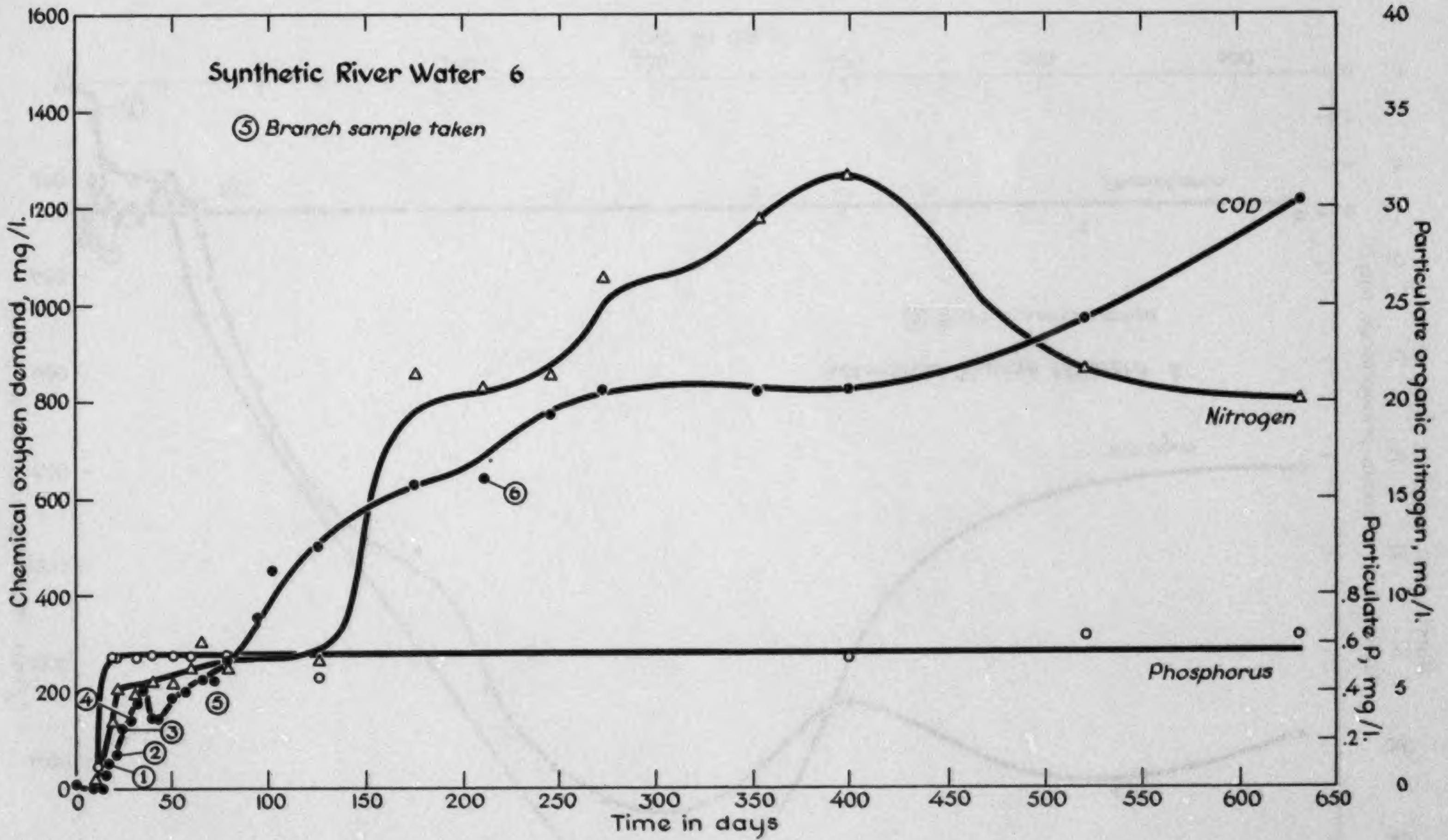

FIGURE 17. Simulated natural algal growth and particulate organic nitrogen and phosphorus concentrations.

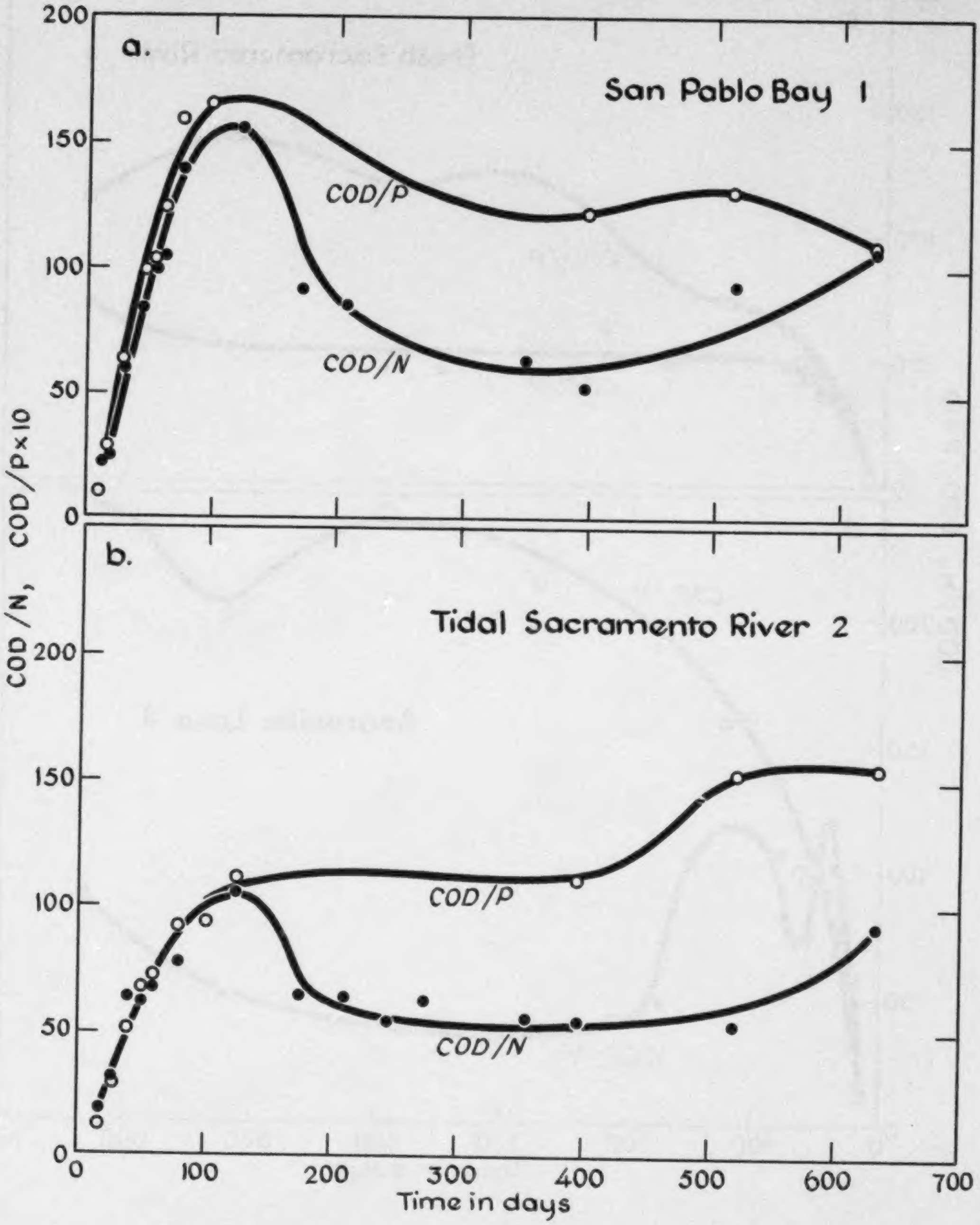

FIGURE 18. A & B. Change of COD/N and COD/P ratios of suspended solids during algal growth.

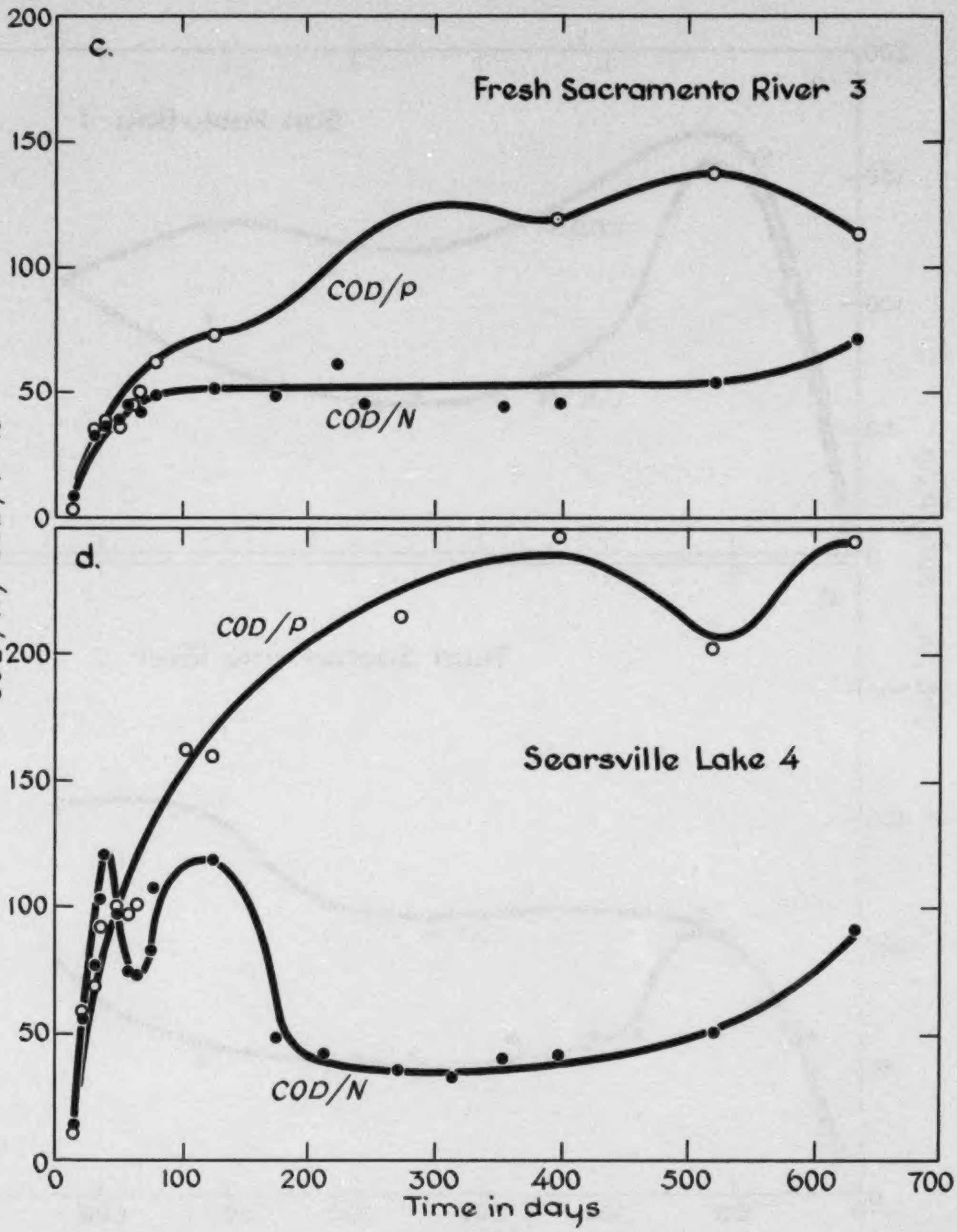

FIGURE 18. C & D. Change of COD/N and COD/P ratios of suspended solids during algal growth.

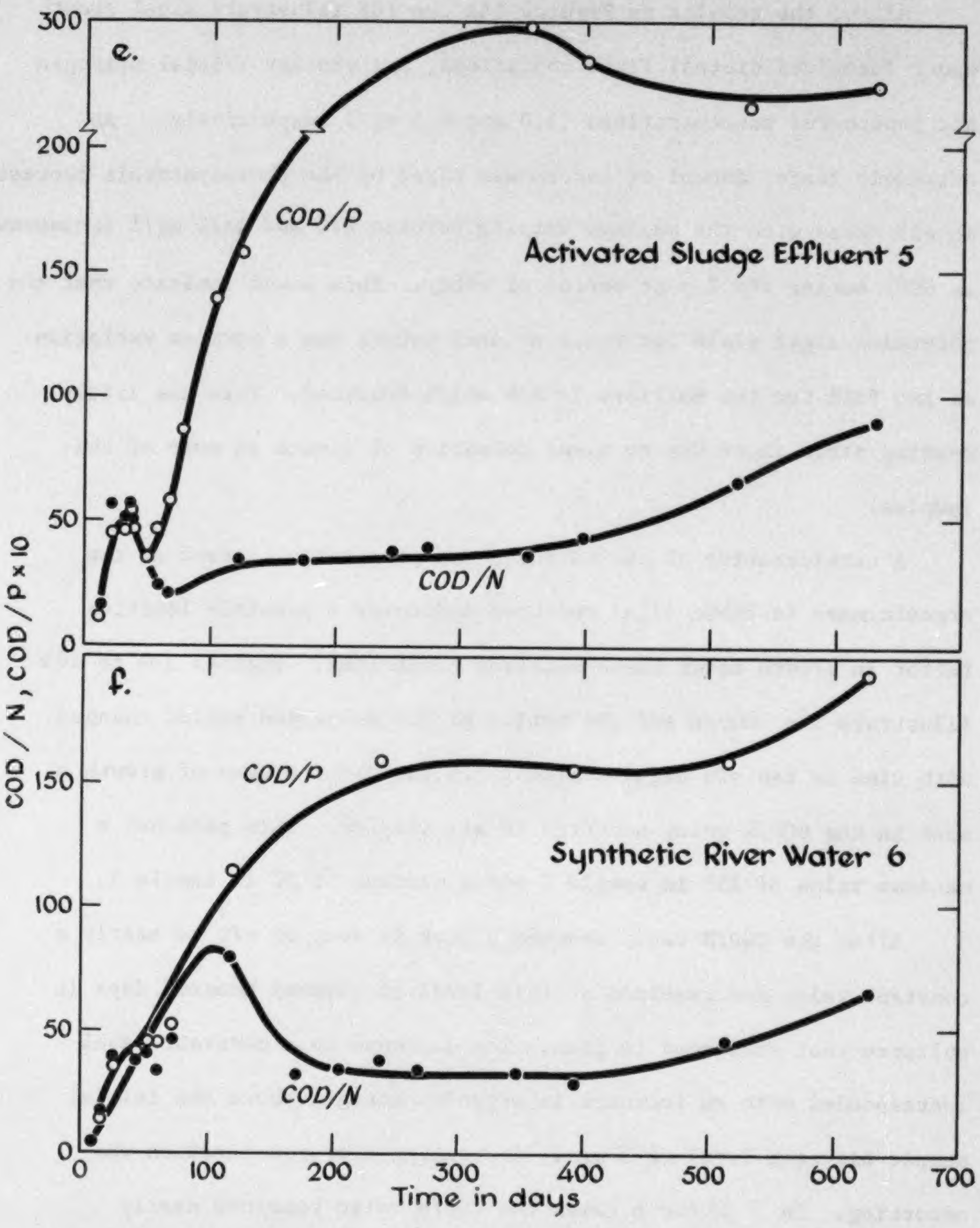

FIGURE 18. E & F. Change of COD/N and COD/P ratios of suspended solids during algal growth.

All of the results in Figures 18A to 18F illustrate algal growth under identical diurnal light conditions, and similar initial nitrogen and phosphorus concentrations (5.0 and 0.5 mg/l respectively). An extremely large amount of carbon was fixed by the photosynthesis process in all cases with the maximum varying between 680 and 1510 mg/l (measured as COD) during the 2 year period of study. This would indicate that the potential algal yield for these natural waters has a maximum variation of two fold for the nutrient levels which occurred. This has little meaning since there was no clear cessation of growth in some of the samples.

A consideration of the nitrogen and phosphorus content of the organic mass in these algal cultures indicates a possible limiting factor in growth under these nutrient conditions. Figures 18A to 18F illustrate the COD/N and the ratios of the suspended solids changed with time in the six algal systems. In the early stages of growth a peak in the COD/N value occurred in all samples. This peak had a maximum value of 155 in sample 1 and a minimum of 50 in sample 3.

After the COD/N ratio reached a peak it dropped off to nearly a constant value and remained at this level of several hundred days in cultures that continued to grow. The decrease to a constant level corresponded with an increase in organic nitrogen above the initial sample nitrogen level of 5 mg/l, indicating nitrogen fixation was occurring. In 5 of the 6 cases the COD/N ratio remained nearly constant between 30 and so,as the COD/P ratio continued to grow larger. Thus the increase in COD was matched by a comparable fixation of nitrogen is shown in Figures 18A to 18F. Microscopic examination indicated that blue-green algal types were present in all samples during the latter stages of growth when nitrogen fixation was occurring. It is significant to note that the large amount of growth was not stimulated by contamination of the samples with phosphorus from the air nor from phosphorus leached from the glass walls of the vessels since the final total phosphorus concentration measured in all samples was within 0.05 mg/l P of the initial ortho phosphorus level given in Table 15.

Table 17 summarizes the variation of nitrogen and phosphorus in the six simulated natural algal culture studies.

Table 17. Variation of Particulate Nitrogen and Phosphorus in Algal Communities.

SAMPLE	MAXIMUM COD/N	NORMAL* COD/N	MAXIMUM COD/P	N/P RATIO INITIAL	N/P RATIO MAXIMUM
San Pablo Bay(1)	155	--	1650	10.0	23.6
Tidal Sacramento River (2)	105	55	1550	6.7	29.7
Fresh Sacramento River (3)	50	50	1350	10.0	28.0
Searsville Lake (4)	120	40	2480	1.8	75.4
Activated Sludge Effluent (5)	90	35	2970	6.4	87.0
Synthe tic River Water (6)	76	30	2400	10.6	60.4

*With nitrogen fixation

The COD/N ratio had a maximum range of 50 to 155 which corresponded with a minimum or limiting nitrogen concentration of 2 to 0.65 per cent (COD basis); whereas the maximum COD/P ratio varied from 1350 to 2970, or a minimum phosphorus concentration of 0.034 per cent. Growth continued in three cases (Samples 1, 5, and 6) at a phosphorus concentration less than 0.041 per cent.

The maximum ratio of the particulate organic nitrogen/particulate organic phosphorus (N/P) was 87 (by weight) with a minimum of less than 2/1. During the initial actively growing stage of most algal systems an N/P ratio of 10/1 was commonly observed.

In all cases of simulated natural growth the same general sequence of biological activity was observed. All natural algal samples had different types of algae with a minimum number of five or six appearing in any one sample. Throughout the course of growth over fifteen morphologically different algae were observed in sample 4. The initial phytoplankton that appeared in all samples were the more delicate flagellated algal forms, diatoms and green algae. As these species reached their stationary phase, new and hardier species of green algae usually became predominant. During this shift in species, a slight color change could be noted from bright green to light green or yellowish color and then back to a dark green. This indicated a general decline in one community with another taking its place almost immediately. This fluctuation in colors occurred several times (as noted by the plateaus in some of the growth curves) with the blue-green algae finally forming the predominant group in the last effort to grow in the nutrient deficient atmosphere.

The plateaus or bumps in the growth curves (sample 3, for example) indicate a cessation of growth of algae and a resulting decline in organic mass. This decline was never accompanied by a significant release of nutrients. The phosphorus concentration was always less than 0.05 mg/1 P in solution even in the stationary phases of samples

1 and 2. Nitrogen was found in solution in some of the cultures with high nitrogen fixation activity. For example, in sample number 5 the total nitrogen content increased from 5 to 43 mg/l due to the fixation process. In these samples ammonia nitrogen increased to several mg/l in solution in samples in the light. After the maximum organic nitrogen concentrations occurred, a rapid decline in the total N concentration was observed in several samples. Since the pH of these samples was observed to be as high as 8.0 during the day, it is felt that the loss of nitrogen occurred as a result of the loss of ammonia due to aeration over a period of months.

Bacterial and animal activity increased up to a certain level and then fluctuated randomly for several months. The microscopic zooplankton went through several blooms with extremely high concentrations of some animals such as the rotifers. In no instance was any animal population apparently ever able to control the phytoplankton growth or to significantly limit the growth potential of the algae. But a leveling off of growth was observed for short periods in the presence of high animal populations in some cases. In most cases the animal population and activity gradually decreased with age until after two years time little activity was noted.

In sample 5 the blue-green algal activity was so intense as to cause a change in the viscosity of the water. The resulting gellatinous character of this culture caused considerable assay problems, especially with filtration.

In all natural algal growth systems the production of extracellular dissolved organic material as measured by analysis of the total organic carbon in the filtrate (using Whatman glass fiber filter, GF/C, 4.25cm) was always less than 20 per cent of the total culture organic carbon, and usually less than 10 per cent. Figure 19 shows the increase in dissolved organic carbon with algal growth in one representative sample. The production of extracellular organic material very likely took place to a significant level in some of the natural algal cultures but bacterial and animal activity apparently kept the concentration at a low level.

Decomposition of Algae

At six predetermined points (shown in Figures 12 to 17) samples were taken from the light cultures and placed in the dark to determine the rate and extent of decomposition. Figures 20 to 25 summarize the data obtained from the decomposition of each branch sample. Because of the variability of microorganisms, water type, and algae studied in this experiment, it might be expected that the biological systems would reflect wide fluctuations in the decomposition results. However, as can be seen in Figures 20 to 25 a fairly smooth and consistent pattern of decomposition was usually observed. All carbon, nitrogen, and phosphorus analyses were conducted on these samples periodically over the complete cycle of decomposition. Table 18 contains a summary of the data for the composition of the algal cultures at the time when the branch samples were first placed in the dark and after decomposition was completed. Two factors are immediately apparent from these results: 1) the natural algae and algal derived organic material decomposed very slowly, and 2) a significant fraction of the initial organic material remained in the systems after stabilization was apparently complete.

In some cases the total COD appeared to increase in the dark before a decrease in the organic matter (as measured by the COD) was

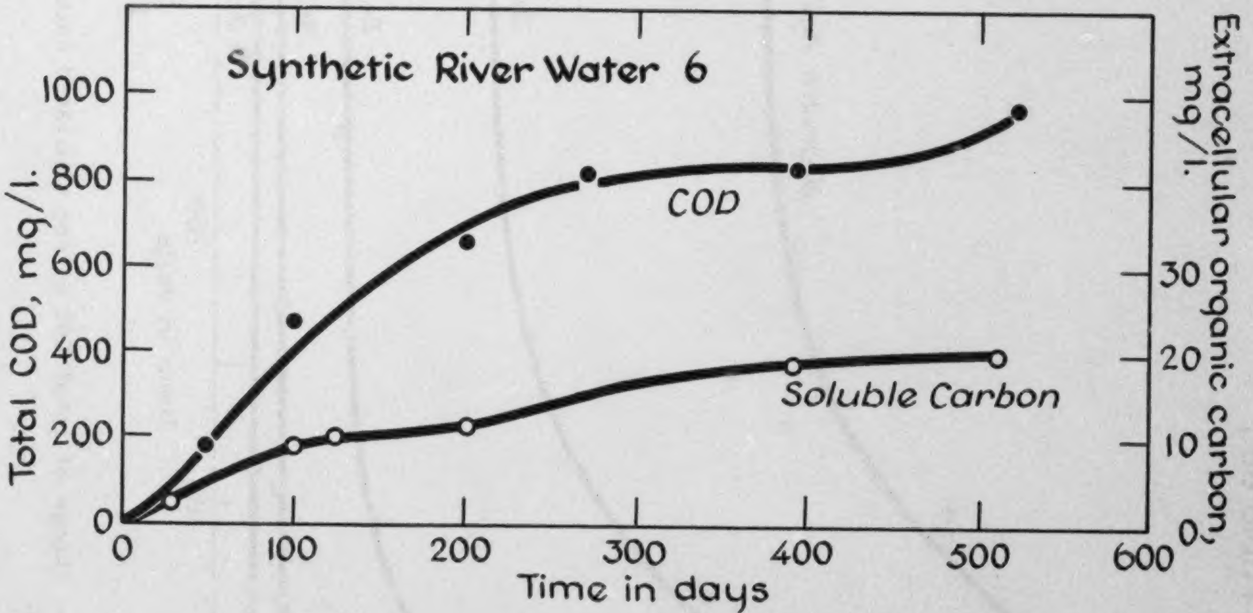

FIGURE 19. Correlation of production of extracellular organic carbon to algal growth.

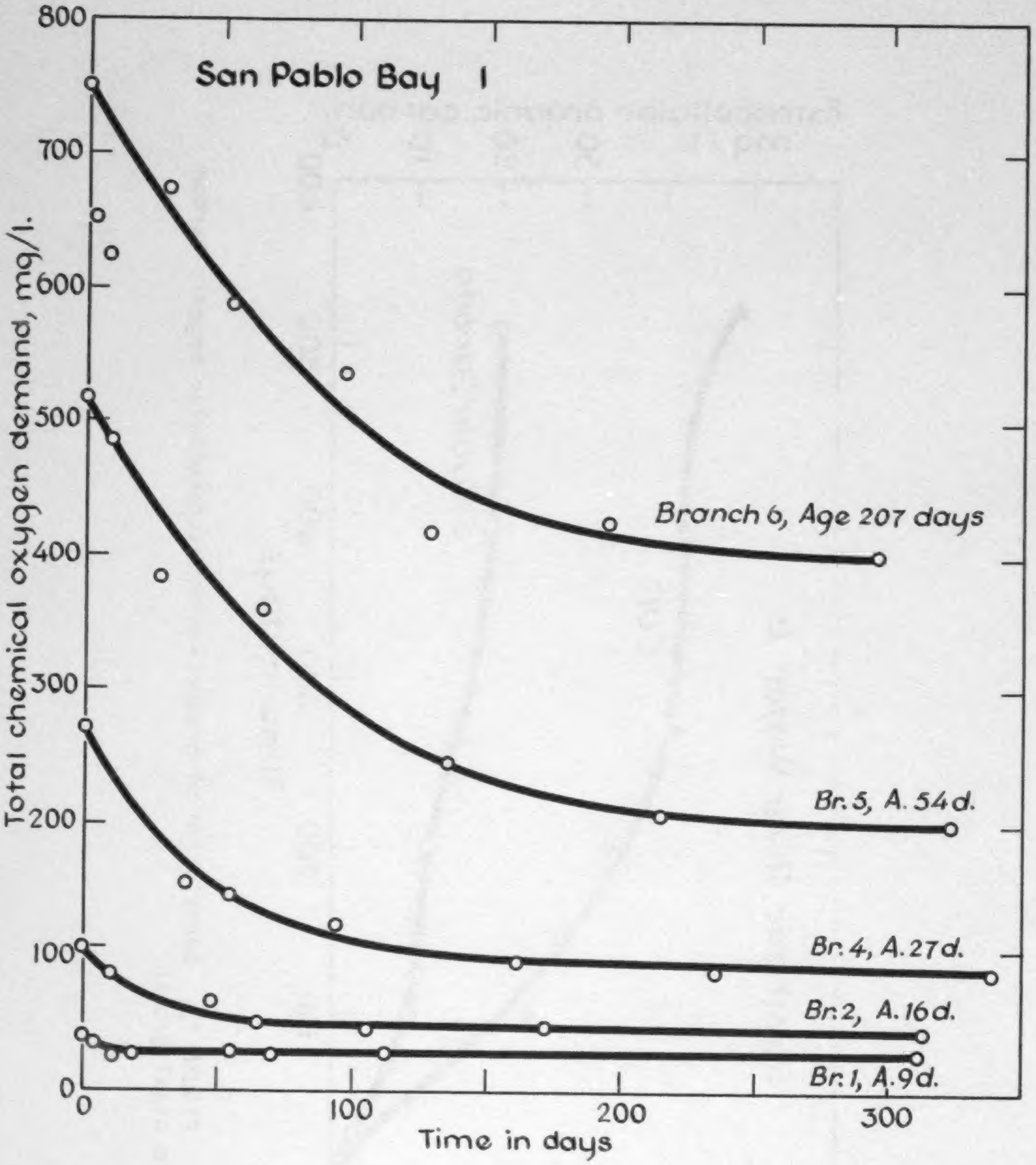

FIGURE 20. Change of total COD during simulated natural algal decomposition.

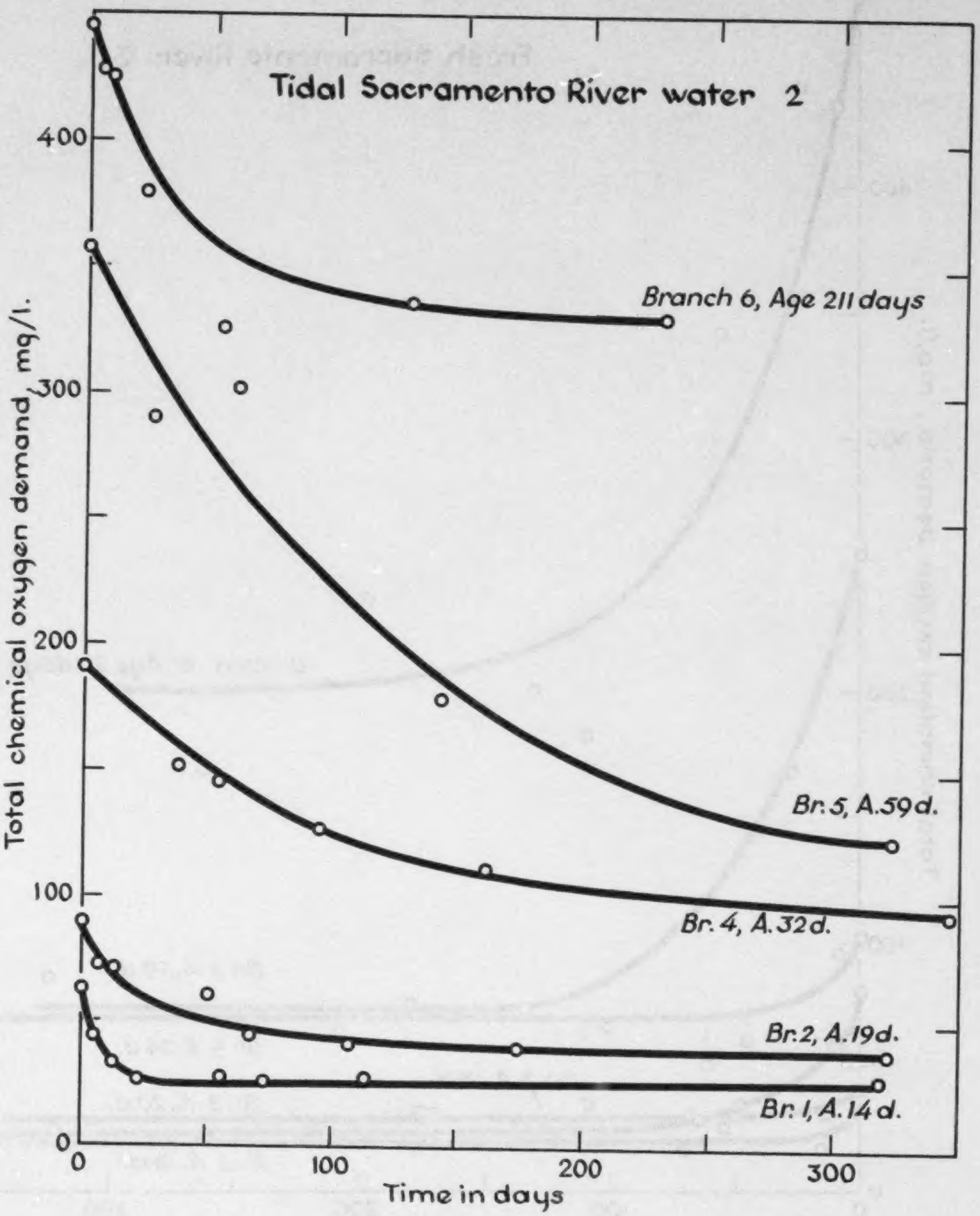

FIGURE 21. Change of total COD during simulated natural algal decomposition.

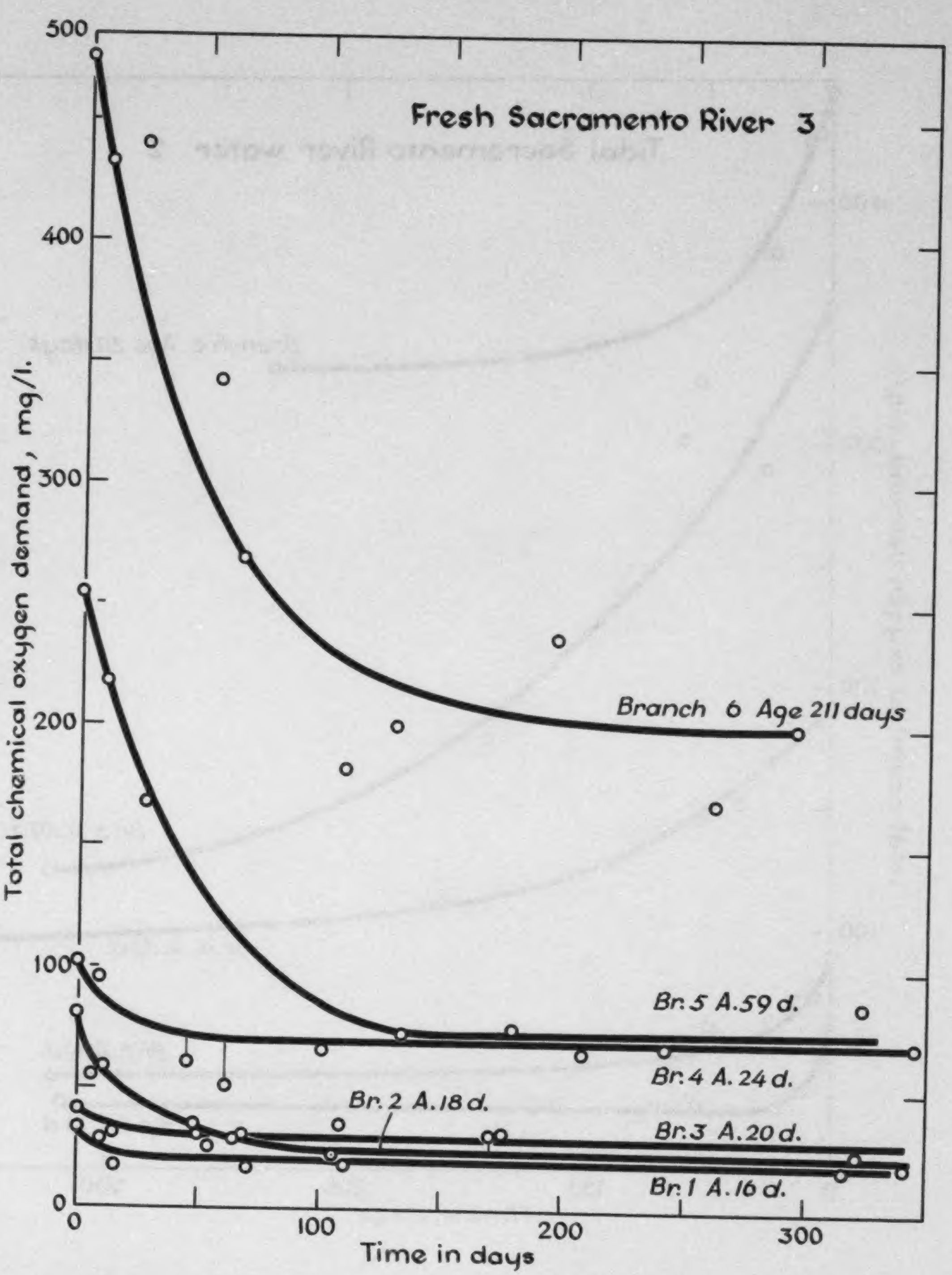

FIGURE 22. Change of total COD during simulated natural algal decomposition.

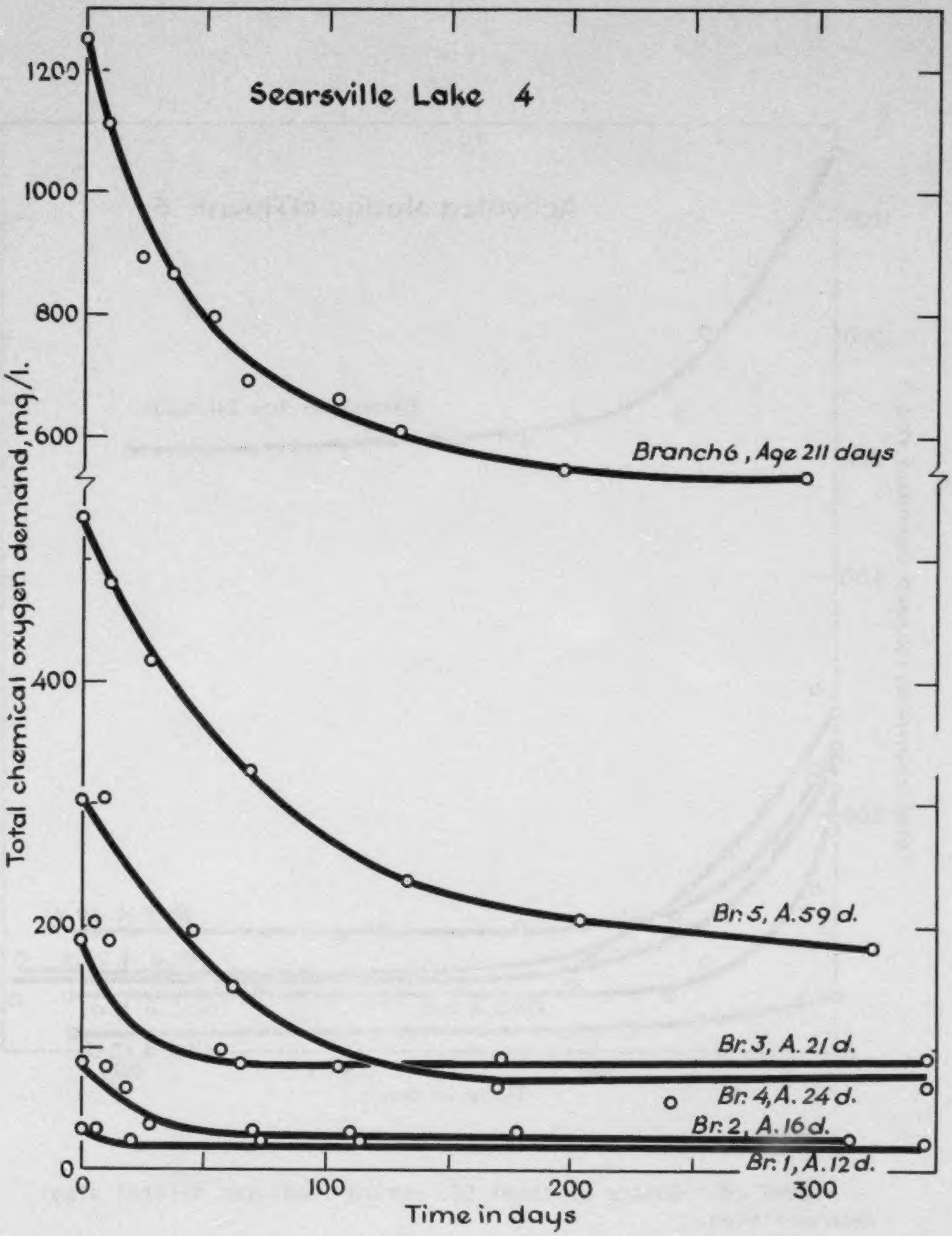

FIGURE 23. Change of total COD during simulated natural algal decomposition.

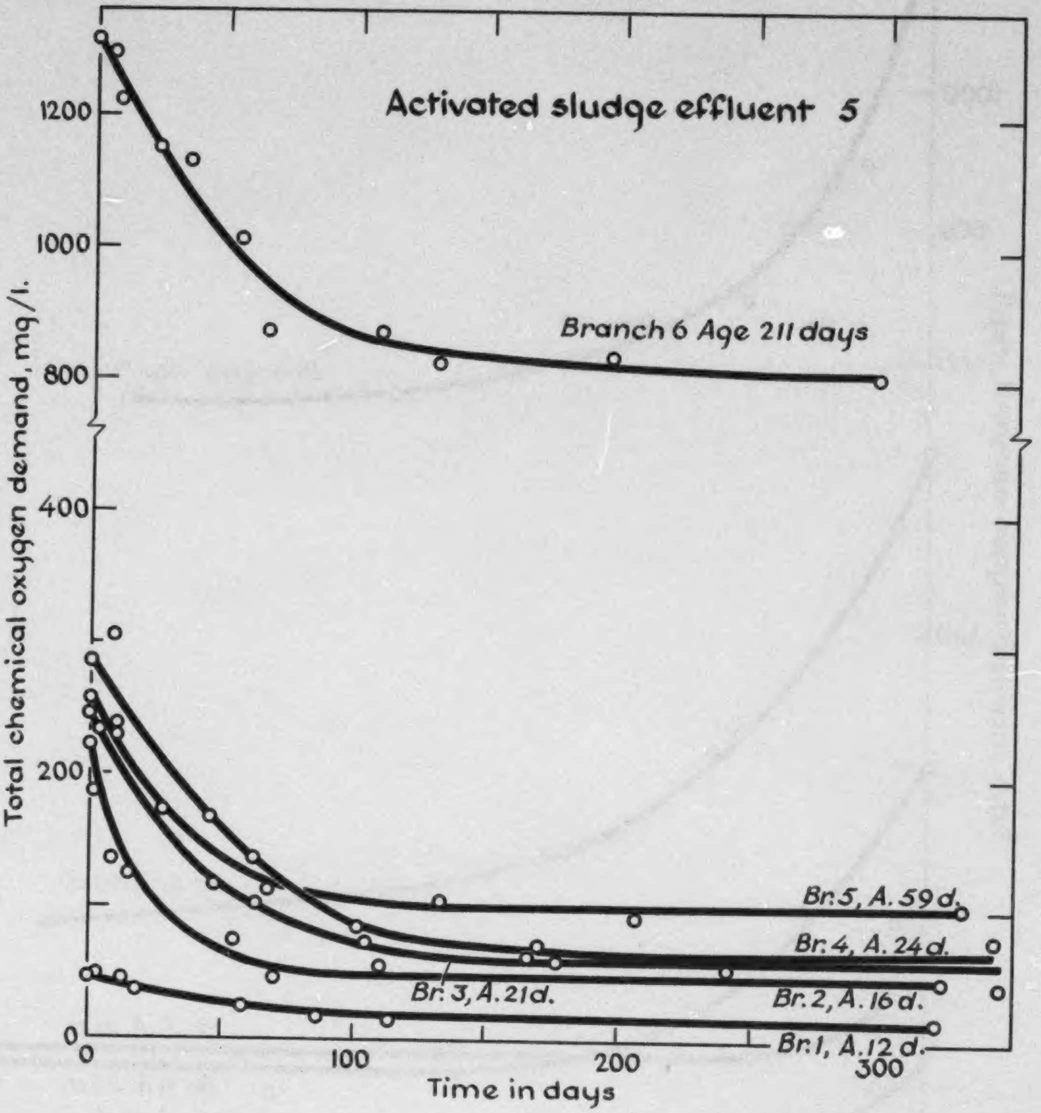

FIGURE 24. Change of total COD during simulated natural algal decomposition.

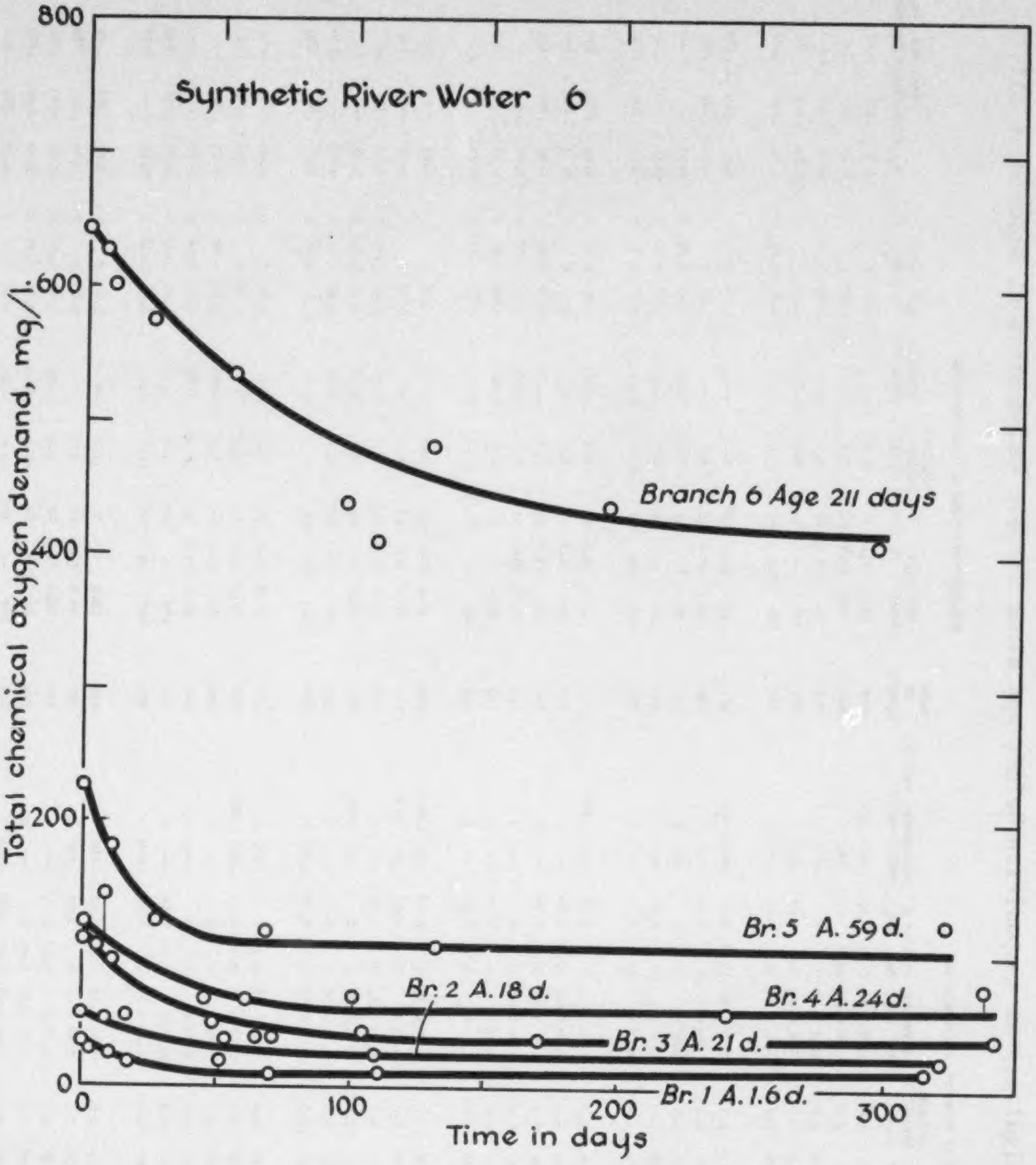

FIGURE 25. Change of total COD during simulated natural algal decomposition.

Table 18. Algal system composition before and after aerobic decomposition, Experiment III.

			COMPOSITION AT START OF DECOMPOSITION											COMPOSITION AT END OF DECOMPOSITION											
			COD, mg/1			FILTATE ORGANIC C	NITROGEN, mg/1 as N				PHOSPHORUS, mg/1 P		DAYS OF DECOMP.	COD, mg/1			FILTRATE ORGANIC C	NITROGEN, mg/1 as N					PHOSPHORUS, mg/1 as P		
SAMPLE	BRANCH	ALGAL AGE (days)	TOTAL	SS	SS	(mg/1)	SS	NH_3	NO_2^-	NO_3^-	TOTAL	ORTHO		TOTAL	SS	SS	(mg/1)	TOTAL ORG.	SS	NH_3	NO_2^-	NO_3^-	TOTAL	TOTAL SOLUBLE	ORTHO
1	1	9	40.0	18.5	32.	5.0	0.90	0.20	0	3.60	.490	.210	312	28.0	9.1	24	5.8	---	0.46	0	0	4.75	.520	.480	.386
1	2	16	108	62.4	90.	2.5	3.00	0.50	0.02	1.00	.500	0	313	42.8	20.2	26	2.6	---	1.60	0	0	3.80	.504	.284	.233
1	4	27	267	220	172	2.3	5.10	0.10	0	0	.500	0	366	90	114	178	5.0	2.10	2.26	0.30	0	2.80	.500	---	.185
1	5	54	518	491	314	8.3	5.20	0.90	0	0.24	.500	0	323	153	---	140	6.8	5.7	4.40	0	0	1.34	.485	.050	.030
1	6	207	749	650	425	9.6	8.70	0	0	0.11	.500	0	295	420	360	218	10.0	---	7.40	0.10	0	0.08	.540	.004	0
2	1	14	62.9	50.0	44	2.3	2.20	0.20	0.09	3.27	.530	.225	317	25.0	10.9	18	5.5	---	0.74	0	0	4.3	.530	.430	.340
2	2	19	80.0	--	65	5.5	2.20	0.15	0	3.00	.500	0	320	38.7	20.7	26	4.0	---	1.56	0	0	3.9	.540	.280	---
2	4	32	191	167	132	12.0	4.00	--	--	0	.500	0	365	93.0	93	102	4.0	2.80	2.80	0.30	0	2.40	---	---	.135
2	5	59	358	319	196	7.0	5.30	0	0	0.18	.510	0	322	123	--	96	5.2	7.60	5.36	2.30	0	0.11	.510	.100	.067
2	6	211	445	415	164	---	5.10	0	0	0.11	---	0	230	330	218	164	11.0	6.95	4.00	2.10	0	0.58	.500	.020	.020
3	1	16	32.9	21	24	2.6	0.90	0.60	0	2.30	.500	.204	315	19.7	13.3	20	2.2	---	0.81	0	0	4.90	.520	.490	.390
3	2	18	41.0	42	40	4.8	3.20	0.50	0.02	1.00	---	0	321	24.9	14.9	22	2.0	---	1.12	0	0	6.10	.540	.450	.280
3	3	20	70.4	68	46	2.9	3.80	0.50	0.10	0.50	---	0	341	18.5	12.7	11	3.2	1.15	1.15	0.02	0	4.45	.498	.415	.240
3	4	24	102	101	99	5.0	5.45	0.30	0	0	---	0	365	71.4	73.3	98	6.0	3.00	3.00	0.30	0	8.40	---	---	.025
3	5	59	254	219	106	7.8	5.60	0	0	0.10	---	0	322	86.4	--	78	6.7	5.80	2.00	0.80	0	2.44	.532	.130	.100
3	6	211	476	--	208	7.0	7.80	0	0	0.20	---	0	295	220	227	156	11.2	11.8	8.52	0.30	0	0.52	---	.067	.020
4	1	12	31.7	21	28	---	1.02	0.25	0	3.00	.600	.230	319	27.3	10.2	22	9.0	---	0.42	0	0	3.60	.550	.416	.370
4	2	16	89.5	--	86	---	5.00	0	0	0.01	.480	.008	313	24.0	12.0	24	6.8	---	0.64	0	0	5.40	.510	.420	.360
4	3	24	185	200	174	7.9	4.16	0.50	0	0.01	---	0	340	91.6	65.8	11	6.8	3.85	2.18	1.10	0	4.10	.492	.300	.250
4	4	31	301	254	221	7.0	5.45	0.20	0	0	.567	.033	365	68.5	53	66	10.0	2.00	2.00	0.50	0	3.45	---	---	.375
4	5	50	534	455	356	9.6	7.15	0.30	0	0	---	0	322	184	---	166	9.6	7.00	5.44	1.30	0	0.69	---	.180	.167
4	6	211	1250	1000	755	12.0	21.7	3.40	0	0.30	.560	0	295	555	555	436	15.8	12.5	11.8	0.80	0	0.84	.690	.190	.015
5	1	12	48.3	42.4	32	6.0	3.00	0.30	0.10	1.30	.600	0	319	17.9	10.9	14	3.1	---	0.36	0	0	3.60	.600	.560	.470
5	2	16	225	186	118	8.5	5.00	0	0.04	0.05	.620	.008	323	49.8	46.4	38	2.5	---	1.72	0	0	5.90	.620	.520	.400
5	3	21	250	214	160	3.3	4.36	0	0	0	---	0	340	46.6	39.6	28	5.0	3.05	1.36	1.80	0	5.02	.563	---	.375
5	4	24	286	260	175	15.0	5.33	0	0	0	.550	0	365	60.0	56.0	50	6.7	1.90	2.26	0.20	0	5.70	---	---	.175
5	5	59	259	216	174	17.4	10.5	0	0.04	0.24	---	0	330	104	--	60	4.0	4.40	3.04	1.00	0	3.90	.887	.370	.333
5	6	211	1250	1200	880	38.0	20.2	0	0	0.36	.580	0	295	800	709	730	19.0	17.0	14.8	1.10	0	0.32	---	.087	.013
6	1	16	36.9	27.0	20	7.0	1.56	0.33	0.01	2.40	.620	.093	315	12.2	7.1	10	1.5	---	0.22	0	0	3.60	.690	.550	.550
6	2	18	54.9	50.0	44	6.8	3.48	0.80	0	0.60	.550	0	321	18.5	13.3	14	0.7	---	0.56	0	0	5.00	.550	.420	.453
6	3	21	75.4	---	70	5.0	5.12	0	0.02	0	---	0	340	38.3	41.7	18	1.8	1.85	1.50	0.10	0	3.30	.550	.418	.420
6	4	24	122	120	96	4.0	5.07	0.40	0.01	0	---	0	365	60	71	52	10.0	2.30	2.00	0.20	0	2.30	.680	.344	.100
6	5	50	222	174	134	6.8	6.15	0.50	0.01	0.06	.550	0	322	100	---	70	5.0	4.20	2.00	0	0	4.00	.520	.330	---
6	6	211	642	---	515	10.0	21.3	0	0	0.10	.550	0	295	430	430	330	13.0	19.6	16.2	1.6	0	0.46	.530	0	0

observed. There are several factors that have the potential of causing an increase in the energy of a closed biological system in the dark. The first mechanism is through the storage of some high energy compound such as ATP or poly phosphates. Although both have the potential of producing further growth, the literature indicates that neither is likely to produce a significant amount (233). Autotrophic mechamisms utilizing reduced ammonia, sulfur, or iron compounds for energy may increase the organic carbon in the dark. However, because of the low concentration of these materials present in this experiment it is not felt that autotrophic production of organic mass was significant. A third possible source of increase in COD could come from the modification of organic material from a form not initially measured by the COD test to one which would be measured. Analytical error might also cause this to appear to be the case. However, this increase occurred quite often in algal cells 20 to 24 days old during the early stages of decomposition, thus indicating that a biological mechanism was responsible for the increase in COD rather than an analytical error. The fact that the young cells dropped from a total COD of 30 to 50 mg/l to 10 to 30 mg/l and remained at this level for periods up to a year indicates that both the experimental set up and the analytical techniques used were adequate for this study.

Table 19 summarizes the refractory organic fraction measured for each branch sample and Table 20 summarizes the initial decomposition rates based on the total organic mass. The refractory fraction in per cent shown in Table 19 was determined by dividing the total COD remaining after stabilization appeared to be complete by the total initial COD and multiplying by 100. The decomposition rates shown

Table 19. Summary of the Refractory Organic Fractions (f) of the Simulated Natural Algal Systems.

SAMPLE	BRANCH	ALGAL AGE (days)	PERIOD OF DECOMP. (days)	INITIAL COD (mg/l)	FINAL COD (mg/l)	f (%)
1	1	9	312	40	28	70.0
1	2	16	313	108	42.8	39.7
1	4	27	366	267	90	33.7
1	5	54	323	518	200	38.5
1	6	207	295	749	420	56.2
2	1	14	317	62.9	25	39.8
2	2	19	320	80	38.7	48.3
2	4	32	365	191	93	48.7
2	5	59	322	358	123	34.4
2	6	211	230	445	330	74.0
3	1	16	315	32.9	19.7	60.0
3	2	18	321	41.0	24.9	61.0
3	3	20	341	70.4	18.5	26.3
3	4	24	365	102	71.4	70.0
3	5	59	322	254	86.4	33.9
3	6	211	295	476	200	42.0
4	1	12	319	31.7	27.3	86.0
4	2	16	313	89.5	24.9	27.3
4	3	21	340	185	91.6	49.5
4	4	24	365	301	68.5	22.7
4	5	59	322	534	184	34.5
4	6	211	295	1250	555	44.4
5	1	12	319	48.3	17.9	37.1
5	2	16	323	225	49.8	22.1
5	3	21	340	250	46.6	18.7
5	4	24	365	286	60	20.9
5	5	59	330	259	104	40.2
5	6	211	295	1258	800	63.7
6	1	16	315	36.9	12.2	33.1
6	2	18	321	54.9	18.5	33.7
6	3	21	340	110	38.3	35.1
6	4	24	365	122	60	49.2
6	5	59	322	222	100	45.0
6	6	211	295	642	410	64.0

in Table 20 were determined as follows: the initial change in COD over a period of between ten and one hundred days (depending on the length of time over which the COD curve could be approximated by a straight line) was divided by the average suspended solids that were measured in the sample over the same time period. This value was then divided by the number of days over which the COD change was measured. Note that these rates are calculated on the total organic mass basis which includes the refractory organic material.

Table 20. Summary of Initial Decomposition Rates Based on the Total Organic Mass (mg COD/mg SS-day)

	BRANCH					
	1	2	3	4	5	6
SAMPLE						
1	.030	.020	---	.039	.019	.006
2	.036	.011	.018	.023	.027	.012
3	.039	---	---	.012	.025	.013
4	.034	.018	.017	.019	.014	.013
5	.017	---	.025	.031	.023	.007
6	.067	.013	.042	.033	.036	.004

A significant and unexpected observation was the relatively high refractory organic fraction in most young algal cultures. The general trend shown in Table 19 with algal cells of 8 to 15 days of age indicates that the refractory fraction was generally greater than 50 per cent. For cells 15 to 59 days of age the refractory fraction usually varied between 30 and 50 per cent, and as the cells aged from 59 to 211 days the refractory organic fraction increased in most cases to more than 50 per cent of that initially present when the sample was placed in the dark. The variation of the refractory organic fraction takes on a pattern when plotted against the age of the algal system. Figure 26 shows this correlation and includes the results from the decomposition studies in this experiment plus nine data points obtained in Experiment IV. Thus it can be seen that although there is a large variation of refractory organic fraction in natural systems, it varies in a defined pattern with algal age.

Note that the three data points falling below the system boundary lines all belong to sample 5. The algae in these branch samples were predominantly blue-green algae and it is believed that the reason for the exceptionally low refractory organic fraction was because of the large production of capsular material by these algae.

Another experiment was conducted to determine if the decomposer seed or the lack of nutrients caused the high refractory organic fraction observed in the oldest branch samples (branch 6). All branches were composited into one sample after decomposition appeared to be complete. This sample was then seeded with the same seed used in Experiment V and nitrogen and phosphorus were added (5 mg/l NO_3^--N and 2 mg/l PO_4^{-3}-P). The sample was then placed in the dark at 20°C and aerated for another 100 days. The resulting change in organic material is noted in Figure 27 and shows that the resulting effect of added nutrients and decomposers was insignificant.

The quantity of refractory organic material formed as the algal system aged is shown in Figure 28 for the six simulated natural algal systems shown in Figures 12 to 17. If these figures are compared

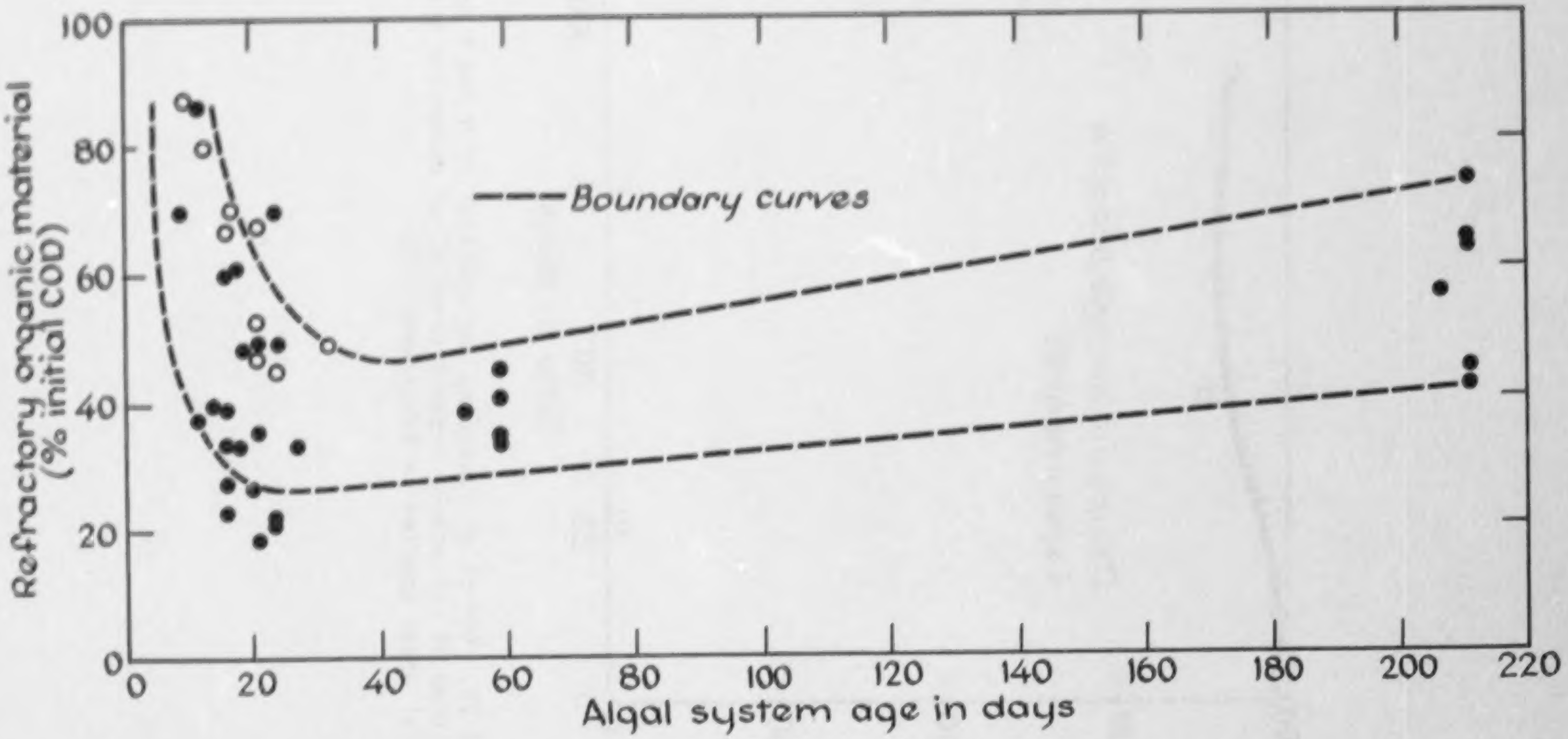

FIGURE 26. Change of refractory organic fraction with algal age.

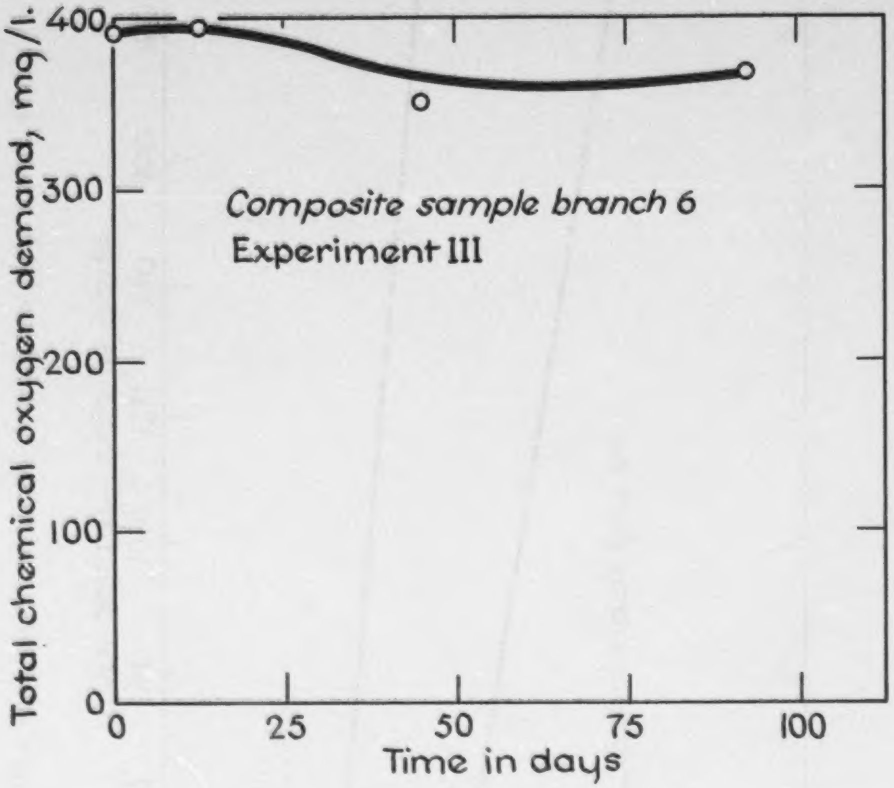

FIGURE 27. Effect of reseeding and addition of N and P on the decomposition of refractory organic material of composite branch 6 of all natural algal samples in Experiment III.

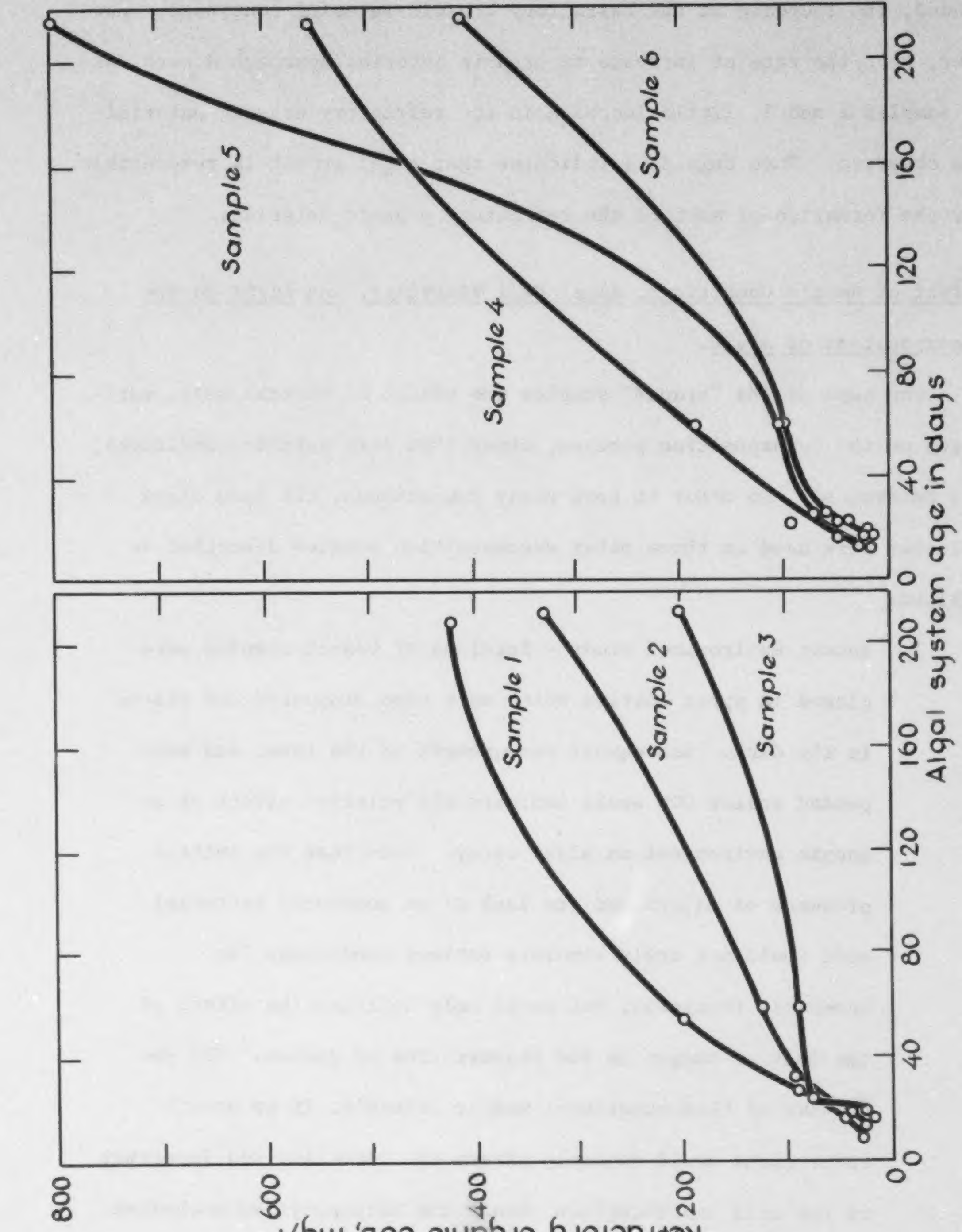

FIGURE 28. Effect of algal age on the quantity of refractory organic material formed.

to the growth figures, it can be seen that as long as growth continued, the increase in the refractory organic material continued. However, when the rate of increase in organic material approached zero, as in samples 2 and 3, little increase in the refractory organic material was observed. Thus this data indicates that algal growth is responsible for the formation of most of the refractory organic material.

Effect of Anoxic Conditions, Algal Cell Viability, and Light on the Decomposition of Algae.

For some of the "branch" samples the effect of several major variables on the decomposition process, other than dark aerobic conditions, was determined. In order to have ready comparisons, the same algal cultures were used in three other decomposition studies described as follows:

1. Anoxic environment study - Portions of branch samples were placed in pyrex bottles which were then stoppered and placed in the dark. Subsequent measurement of the total and suspended solids COD would indicate the relative effect of an anoxic environment on algal decay. Note that the initial presence of oxygen and the lack of an anaerobic bacterial seed would not truly simulate optimum conditions for anaerobic treatment, but would only indicate the effect of the lack of oxygen on the regeneration of carbon. The objective of this experiment was to determine if an anoxic environment would severely affect the viability and integrity of the cell and therefore change the decomposition mechanism.
2. Fractionated Algae Studies. In order to determine the difference between live and dead algal decomposition, branch cultures

were disintegrated and then aerated in the dark. Disintegration was obtained by using a French pressure cell (Model 12-105, American Instrument Co., Inc.) using the following procedure: a 250 ml algal sample was concentrated into a volume of about 10 ml and then placed in the stainless steel French pressure cell fitted with a plunger. The plunger was used to apply 10,000 psi pressure to the liquid volume by means of a hydraulic manually operated lever. The pressure was increased to 10,000 psi three times and released practically instantaneously. In the final step the algal sample was allowed to escape through a small spiget in the bottom of the pressure cell at a rate not exceeding 2 ml per minute as the pressure was maintained at 10,000 psi. The forcing of the algal material through this opening at these pressures served to crush and tear any large particulate material that remained. Microscopic observation indicated that the algal cells had been broken into unidentifiable organic particles by this procedure, approximately 100 to 1000 times smaller than the original cell.

3. Dark and Light Decomposition. In order to determine the effect of a short exposure to light upon algal decomposition, samples of algae were aerated in the dark and exposed to 30 minutes of light per day.

The main branch samples in total darkness received considerably more attention than did those from the side experiments described above. The total and soluble COD was the only variable studied in the anoxic, fractionated, and dark and light decomposition studies.

Effect of an Anoxic Environment on Decomposition. At the same time branch algal samples were taken from the natural algal growth vessels some of these samples (corresponding to branches 1, 2, 5, and 6) were tightly stoppered in decomposition vessels and placed in the dark to ascertain the effect of lack of oxygen on the regeneration of algal organic carbon. The summary of the results in Table 21 shows that anoxic conditions did not significantly affect the quantity of algal material degraded. The total COD reduction is given for the aerobic study and compared to the suspended solids COD reduction in the anoxic conditions. Since the soluble organic matter in the aerobic decomposition studies was usually less than 10 per cent of the total organic matter these two values yield a valid comparison of carbon regeneration. With younger cells the per cent of reduction was about the same in both conditions, whereas with older cells reduction under anaerobic conditions was usually less. With younger samples with a lower concentration of organic matter in the stoppered bottles there was little accumulation of soluble material and thus the reduction of the suspended solids was approximately the same as that in the aerated samples. However, in the older branch samples (5 and 6) the summation of the soluble and particulate COD was nearly equal to the initial total COD. Thus the difference in decomposition in the older cells might be accounted for by lack of an anaerobic seed.

This short study was undertaken to determine if perhaps anoxic conditions might kill the cells or cause them to lyse so that they would be more susceptible to decomposition. These cursory results indicate that the quantity of algae decomposed under anoxic conditions might be

Table 21. Effect of Anoxic Conditions on the Refractory Organic Fraction of Algae.

SAMPLE	BRANCH	ALGAL AGE (days)	AEROBIC DECOMPOSITION IN 300 DAYS (% COD Remaining)	ANAEROBIC DECOMPOSITION IN 300 DAYS (% COD_{ss} Remaining)
1	1	9	75	70
1	2	16	40	41.5
1	5	54	34.8	73.5
1	6	207	63	77.5
2	1	14	61.8	47.8
2	2	19	51.5	62.5
2	6	211	67.5	84
3	1	16	59.8	45.4
3	2	18	60.6	57
3	5	59	27.4	39.5
3	6	211	42	61
4	1	12	86	58
4	2	16	27.8	25.3
4	5	59	34.5	53.4
4	6	211	44.3	60.0
5	1	12	37.1	60.8
5	2	16	22.2	26.8
5	5	59	40	54
5	6	211	66	76.4
6	1	16	33	100
6	2	18	31	39
6	5	59	54	58.5
6	6	211	64	81.8

similar to that under aerobic conditions. Because of the prevalence of anoxic conditions in all areas of the natural environment, this problem remains a fertile area for future work.

Live versus Dead Algal Decomposition. The decomposition of algal cells killed by disintegration was compared to natural aerobic decomposition of live cells. This test was performed on branch samples 1,2, 5, and 6 of all natural waters in this experiment. Typical results are shown in Figure 29. In most cases disintegration of the cell significantly increased the rate of decomposition, but had little effect on the refractory cell material. This study indicates that the integrity of the cell is a major factor in controlling the rate of decomposition of algae. It is felt that the results have little practical application, since the method used to kill the cell was drastically different than conditions which might occur naturally. However, two cases to which this might apply are as follows: 1) when algae are killed by a pollutant, and 2) when algae are killed by chlorination of an algal pond effluent. These possibilities indicate that further research on the effect of various conditions on algal cell viability and the effect of viability on the decomposition mechanism would be worthwhile.

Another practical application of the disintegration method used in this short study would be for the rapid determination of the biodegradable fraction of algae.

Light versus Dark Decomposition. Three branch samples (corresponding to branches 1, 2 and 5) were taken from each one of the simulated natural algal communities and placed in the dark under similar conditions as the samples in complete darkness, except they were exposed to 30 minutes of illumination (300 foot-candles) each day. This appeared to

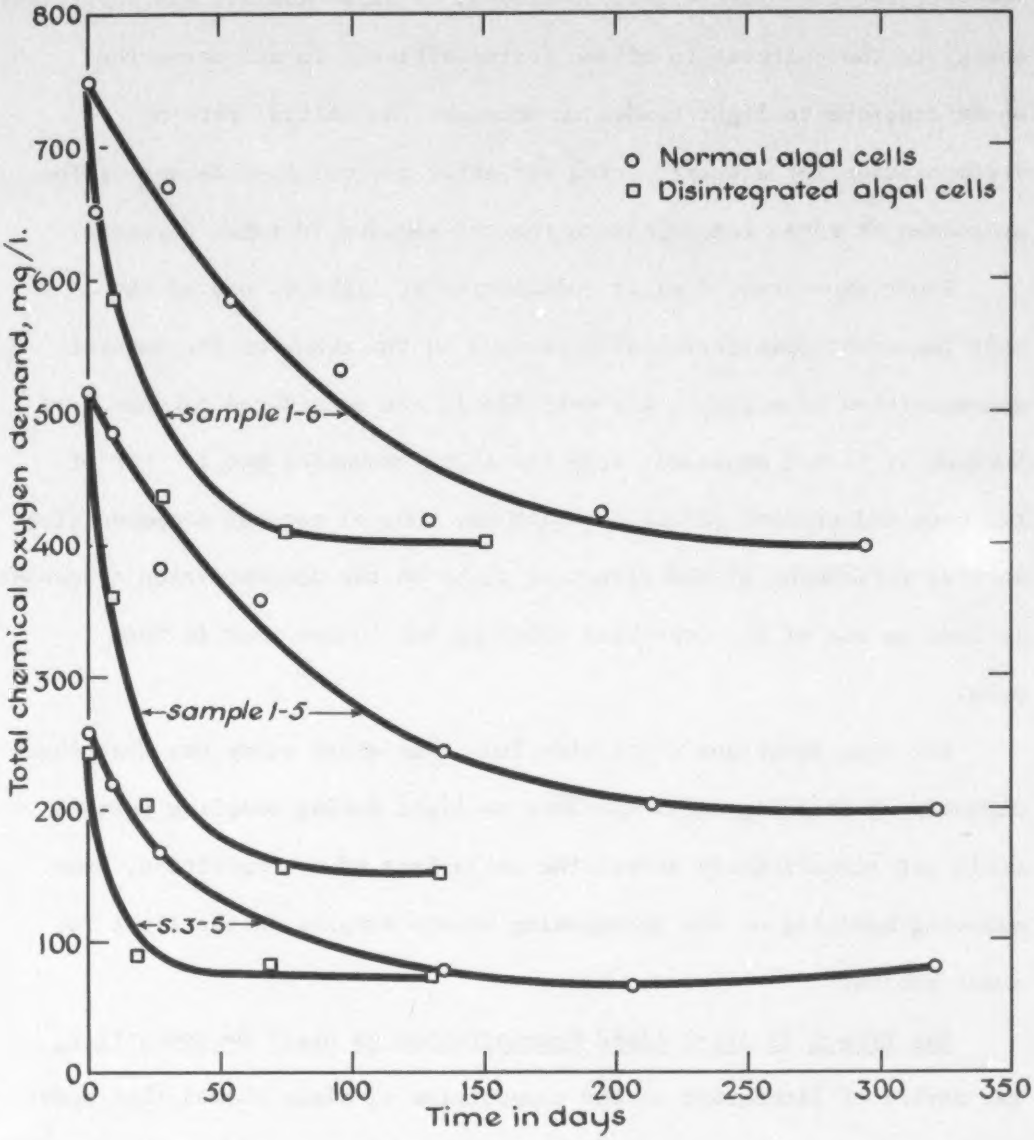

FIGURE 29. Comparison of the decomposition of live and disintegrated algal cells.

inhibit decomposition during the early stages with the young cells. However, for the older algae, 30 minutes of light did not add sufficient energy to the cultures to offset decomposition. In all cases the short exposure to light tended to decrease the initial rate of decomposition for a short period but after several days decomposition proceeded at rates comparable to that of samples in total darkness.

Short exposures of algal communities to light is one of the most important practical considerations in the study of the natural decomposition of algae. This variable is not considered further here because it is not dependent upon the algal community but is one of the many independent variables which may control natural decomposition. Further refinement of the effect of light on the decomposition processes is left as one of the important subjects for future work in this area.

The most important conclusion from this short study was that short exposure of decaying algal cultures to light during sampling periods would not significantly affect the mechanisms of decomposition, thus allowing handling of the decomposing branch samples in the light for short periods.

The Effect of Algal Lipid Concentration on Algal Decomposition. The review of literature on the composition of algae showed that under certain circumstances lipid type material may compose up to 80 per cent of the dry weight of algal material (30). For this reason, a short study of the formation and decomposition of lipid material (total chloroform extractables) in algal cells was included. Table 22 summarizes the significant results of this study. Some of the older branch samples

Table 22. Change in Algal Lipid Concentration During Decomposition.

		INITIAL MEASUREMENT				FINAL MEASUREMENT			
SAMPLE	BRANCH	PERIOD OF DECOMP. (days)	SUSPENDED SOLIDS (mg/l)	LIPID CONCENTRATION (mg/l)	%SS	PERIOD OF DECOMP. (days)	SUSPENDED SOLIDS (mg/l)	LIPID CONCENTRATION (mg/l)	% SS
1	6	79	320	136	42.6	232	254	31.5	12.4
2	6	68	200	111	55.0	231	168	28.0	16.7
3	6	69	186	92	49.5	232	143	16.0	11.2
4	6	69	575	76	13.2	232	400	13.0	3.2
5	6	78	740	52	7.0	228	750	29.5	3.9
6	6	74	400	51	12.7	232	296	24.5	8.2

after decomposing for over 70 days in the dark had a lipid content of up to 55 per cent. After the algal systems were nearly completely stabilized the highest concentration of lipid in all six samples was 16.7 per cent with the lowest at 3.5 per cent. Several factors are significant in these results. First, it should be noted that lipid accumulation much above ten per cent only occurred in those samples where little nitrogen fixation had taken place. This supports the work of many others which indicates that lipid accumulation is stimulated by nitrogen deficiency. Secondly, it can be seen in Table 22 that nearly all the decomposition that took place in samples 1-6, 2-6, and 3-6 between days 70 and 230, can be accounted for by a decrease in lipid material. Even in very old cells it would appear that most of the lipid material was biodegradable. It might be expected that the algal systems that accumulated lipids would have a refractory organic fraction less than samples that did not. As can be seen in Table 19, this was not the case. In future research on the determination of the properties and the formation of the refractory organic material this may be one of the subjects which should receive careful attention.

Nitrogen and Phosphorus Regeneration

The conversion of particulate organic nitrogen and phosphorus to soluble forms during the simulated natural algal decomposition studies are shown in Figures 30 and 31. Note that in most instances nutrient regeneration will be represented by the change in the particulate nitrogen and phosphorus rather than the change in the soluble material.

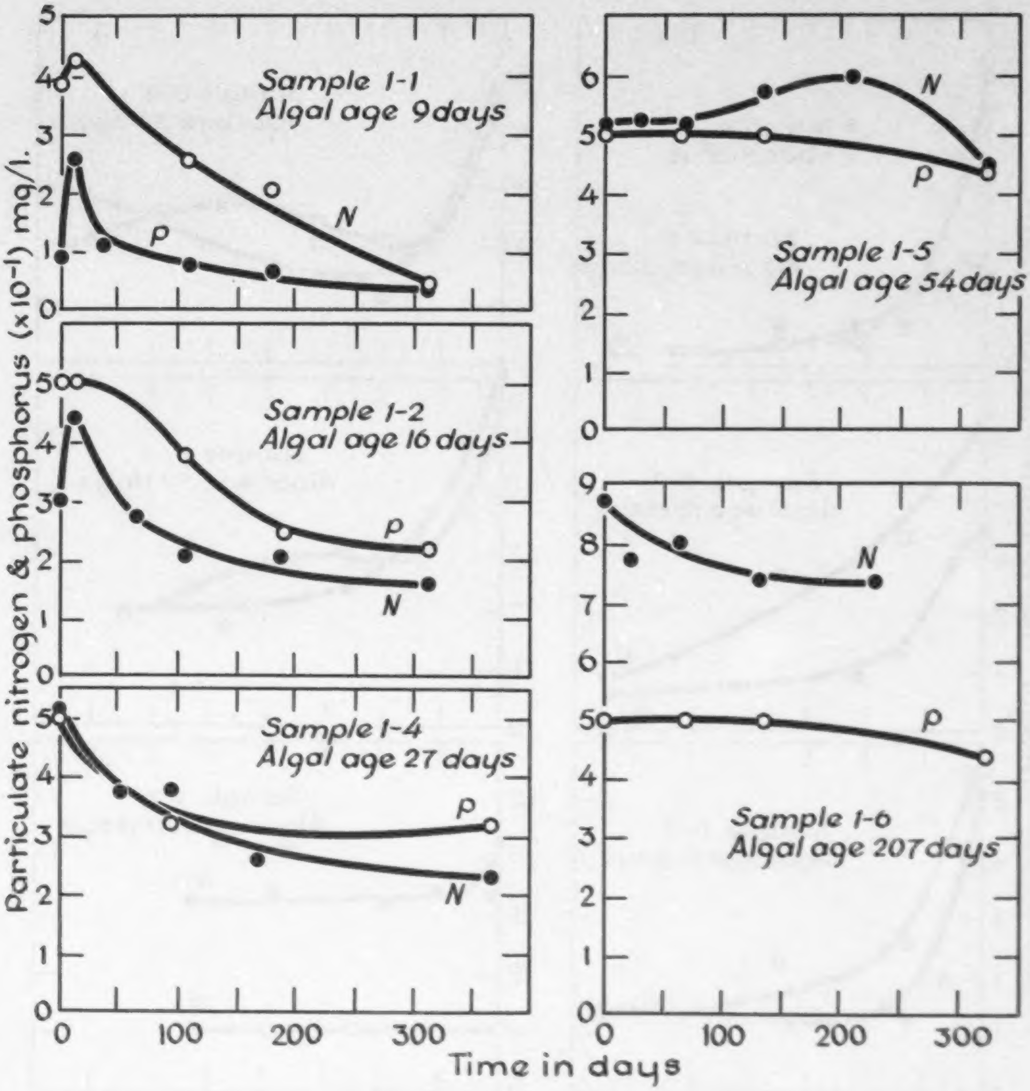

FIGURE 30. Change of particulate N and P as a result of nutrient regeneration during aerobic decomposition of sample 1.

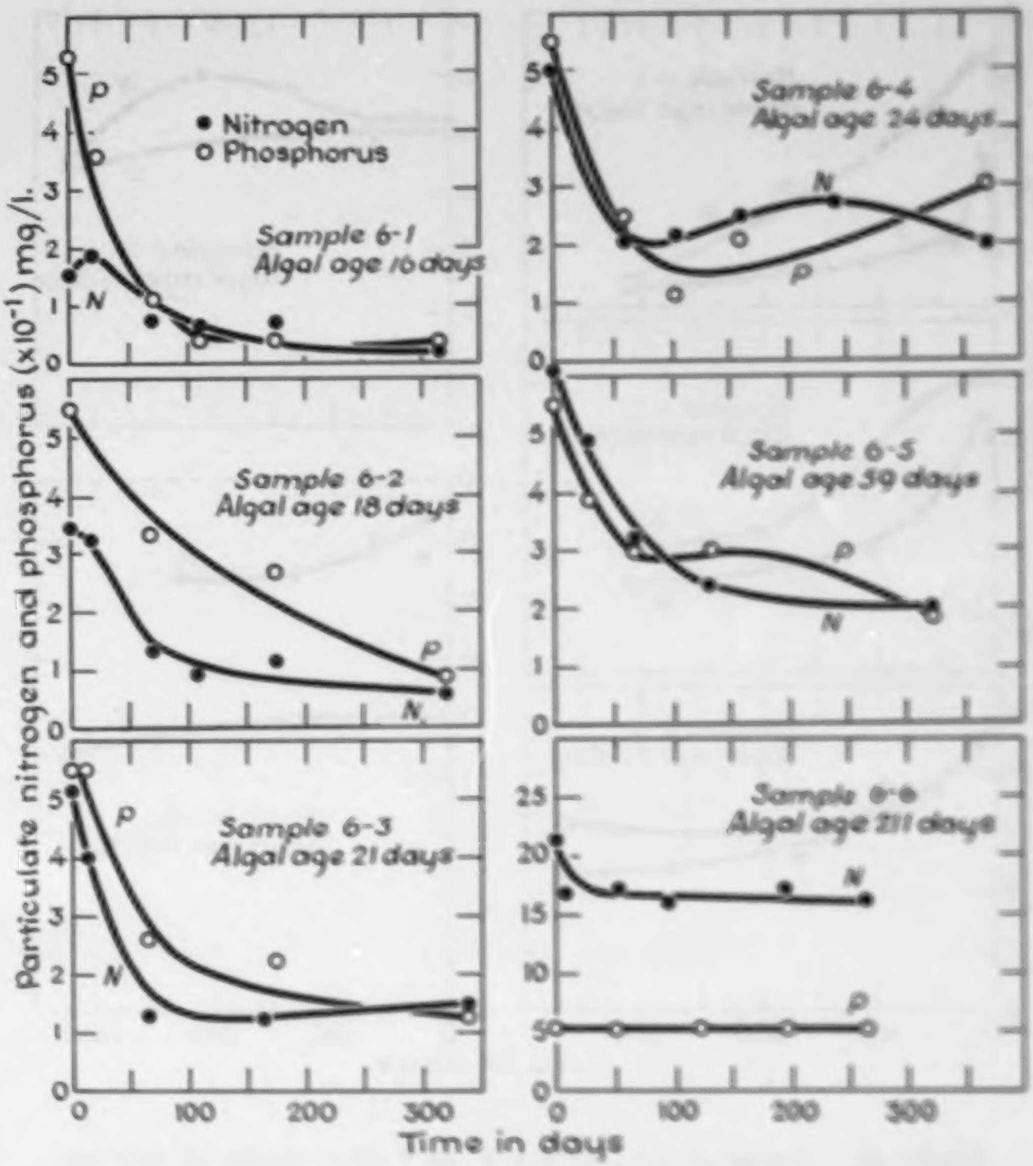

FIGURE 31. Change of particulate N and P as a result of nutrient regeneration during aerobic decomposition of sample 6.

This was done because it was felt that particulate organic nitrogen was a more accurate measurement than the sum of all the soluble forms. These figures may be taken as representative of all the regeneration studies observed in this experiment. Figure 30 represents regeneration in a sample (1) where little nitrogen fixation occurred and where all the branch samples corresponded well to all phases of algal growth ranging from the logarithmic to the death phase. Figure 31 represents regeneration in a case where the nitrogen concentration was increased to greater than 400 per cent of the initial value due to nitrogen fixation. Almost all zones of nutrient regeneration illustrated in Figure 5 in the Background Section were observed in this experiment. The only factor not observed was a rapid regeneration of nutrients during the first few days in the dark. No measurements were made until after 5 or 10 days of decomposition had occurred. In the young samples an increase in particulate nitrogen between 0 and 100 per cent of the initial value occurred as might be expected in zone A. The increase in particulate phosphorus in zone A was much less. In branch samples 1 and 2 of samples 2, 3, 4, and 5, zone A and B type regeneration was not observed, and regeneration appeared to start as soon as the samples were placed in the dark. In the branch samples 3, 4, 5, and 6 zone B type regeneration increased in length from zero to the entire length of the study.

As would be expected based on the decomposition results, algal system age also exerted a controlling influence on nitrogen and phosphorus regeneration. In fact, the effect of the age on nutrient regeneration is much more significant than the similar effect on carbon decomposition. Nearly 100 per cent of the nitrogen and phosphorus was released in older algal cultures. Table 23 summarizes the concentration of nitrogen and phosphorus (as ratios of COD/N and COD/P) in the algal material at the beginning and end of the decomposition period, plus the percentage of the initial particulate nitrogen and phosphorus that was regenerated. The percentage of nitrogen and phosphorus in R varies from 1.5 to 6.2 and 0.067 to 1.5; with average values of 2.7 and 0.25 per cent respectively. The dependence of nitrogen and phosphorus regeneration on algal age and the initial nitrogen and phosphorus concentration are shown in Figures 32 and 33 . The curves in these figures are lines of best fit drawn by eye. In both cases it can be seen that a better correlation was obtained with the percentage of phosphorus regenerated than with nitrogen regeneration. This is probably due to the fact that the nitrogen concentrations varied over a wide range and usually increased with age (total concentration) causing some regeneration to occur even in some very old cultures; whereas the maximum phosphorus concentration was always fixed at approximately 0.3 mg/l P. Note that in algal cells 13 to 20 days old regeneration was as high as 89 per cent for nitrogen and 94 per cent for phosphorus. As the algal cells aged the amount regenerated decreased until at age 211 days less than 3 per cent of the phosphorus was regenerated in all samples. Nitrogen regeneration at 211 days of algal age varied from zero to 46 per cent. Although many variables affect regeneration Figure 33 shows a close correlation between the initial nitrogen and phosphorus concentration in the algal material and the per cent which is regenerated. Note that as the COD/N and COD/P ratios approach 100 and 1000 respectively, the quantity of

Table 23. COD/N and COD/P at the Beginning and End of Decomposition and Per Cent Regeneration in Experiment III.

SAMPLE	BRANCH	ALGAL AGE (days)	PERIOD OF DECOMP. (days)	COD/N		PER CENT N REGENERATED	COD/P		PER CENT P REGENERATED
				INITIAL	FINAL		INITIAL	FINAL	
1	1	9	312	44.9	61.0	49	105	700	90
1	2	16	313	36.0	26.7	46	216	194	56
1	4	27	366	52.4	38.1	55	534	286	37
1	5	54	323	117	34.8	15	1030	352	13
1	6	207	295	86	56.7	15	1500	840	0
2	1	14	317	28.5	33.8	66	206	250	70
2	2	19	320	36.3	24.8	29	153	161	54
2	4	32	365	46.8	33.2	30	383	255	27
2	5	59	322	67.5	22.9	0	702	300	20
2	6	211	263	87.2	40.0	21	890	333	4
3	1	16	315	36.7	24.3	11	104	66	90
3	2	20	321	12.8	22.2	65	76	277	74
3	3	18	341	18.5	16.1	70	141	217	83
3	4	24	365	18.7	23.8	45	204	150	5
3	5	59	322	45.4	43.1	64	480	215	24
3	6	211	295	61.0	25.8	0	950	460	4
4	1	12	319	31.0	65.0	59	93	204	61
4	2	16	313	17.9	38.8	87	180	277	82
4	3	24	340	44.4	42.0	48	370	476	62
4	4	31	365	55.3	34.2	63	580	183	27
4	5	59	322	74.7	33.8	24	1030	543	34
4	6	211	295	57.4	47.0	46	2270	1035	3
5	1	12	319	16.1	49.7	88	81	447	93
5	2	16	323	45.0	29.0	66	409	411	78
5	3	24	340	57.5	34.2	69	434	266	68
5	4	21	365	53.7	26.5	58	320	160	32
5	5	59	330	63.2	34.2	50	472	480	60
5	6	211	295	62.2	34.0	27	2280	1490	3
6	1	16	315	23.6	30.4	86	70	406	94
6	2	18	321	13.7	33.0	84	100	336	85
6	3	21	340	16.7	23.5	71	157	293	76
6	4	24	365	24.1	30.0	61	222	200	43
6	5	59	372	36.1	30.0	67	403	327	65
6	6	211	295	30.1	26.6	24	1170	780	0
			Average		34.0			399	

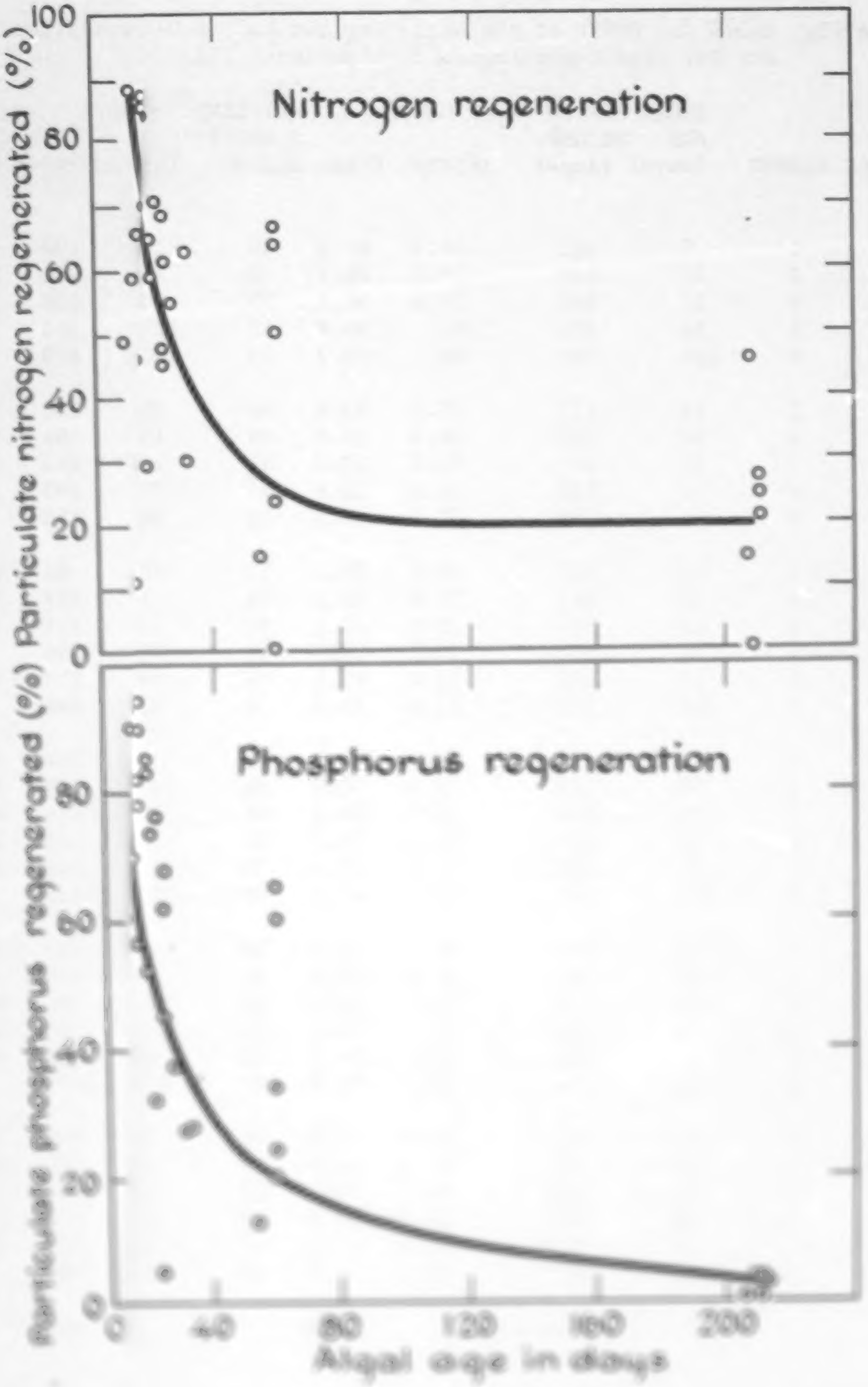

FIGURE 1 Effect of algal age on per cent particulate nitrogen and phosphorus generated during the decomposition of algal.

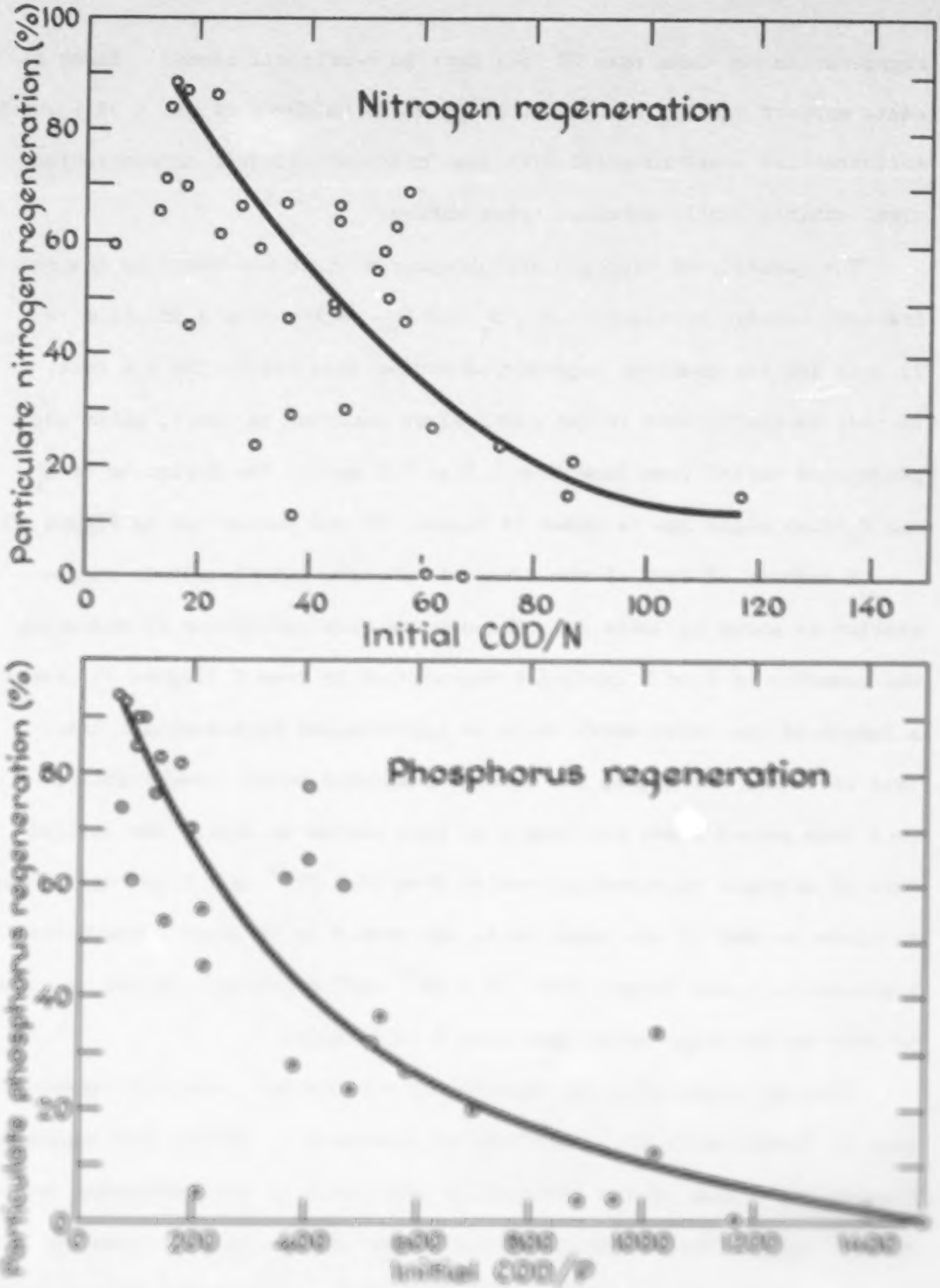

FIGURE 33. Effect of initial nitrogen and phosphorus concentration on the per cent regenerated during decomposition (Experiment III).

regeneration was less than 20 per cent in nearly all cases. These results support results such as those given by Goldbery et al. (98) which indicate that algal material with the "minimum" nutrient concentration might exhibit little nutrient regeneration.

The quantity of nitrogen and phosphorus which was bound in the refractory organic material, i.e., N_r and P_r, varied from a fraction to 15 mg/l (as the quantity regenerated varied from nearly 100 per cent of that initially held in the particulate material to zero), while the phosphorus varied from less than 0.1 to 1.0 mg/l. The formation of N_r and P_r with algal age is shown in Figure 34 and summarized in Figure 35 .

A summary of some of the rates of nitrogen and phosphorus regeneration is shown in Table 24. These rates were calculated by measuring the quantity of N or P initially regenerated in zone C (Figure 5) over a length of the curve which could be approximated by a straight line. This value was divided by the average suspended solids concentration over this period times the length of this period in days. The initial rate of nitrogen regeneration varied from 64 X 10^{-4} mg/l N per mg/lSS-day to close to zero as the algal cells age from 9 to 39 days. Phosphorus regeneration rates ranged from 778 X 10^{-6} mg/l P per mg/l SS-day to close to zero as the algal cells aged from 9 to 32 days.

Thus by considering the variable of culture age, nutrient regeneration is indicated to be a very complex phenomenon. Rather than regeneration of two thirds of the nitrogen or phosphorus or any particular fraction of the substance initially held in the organic mass as commonly reported in the literature, the quantity varied from nearly 100 per cent in young algal systems to zero per cent in older systems. The rate of

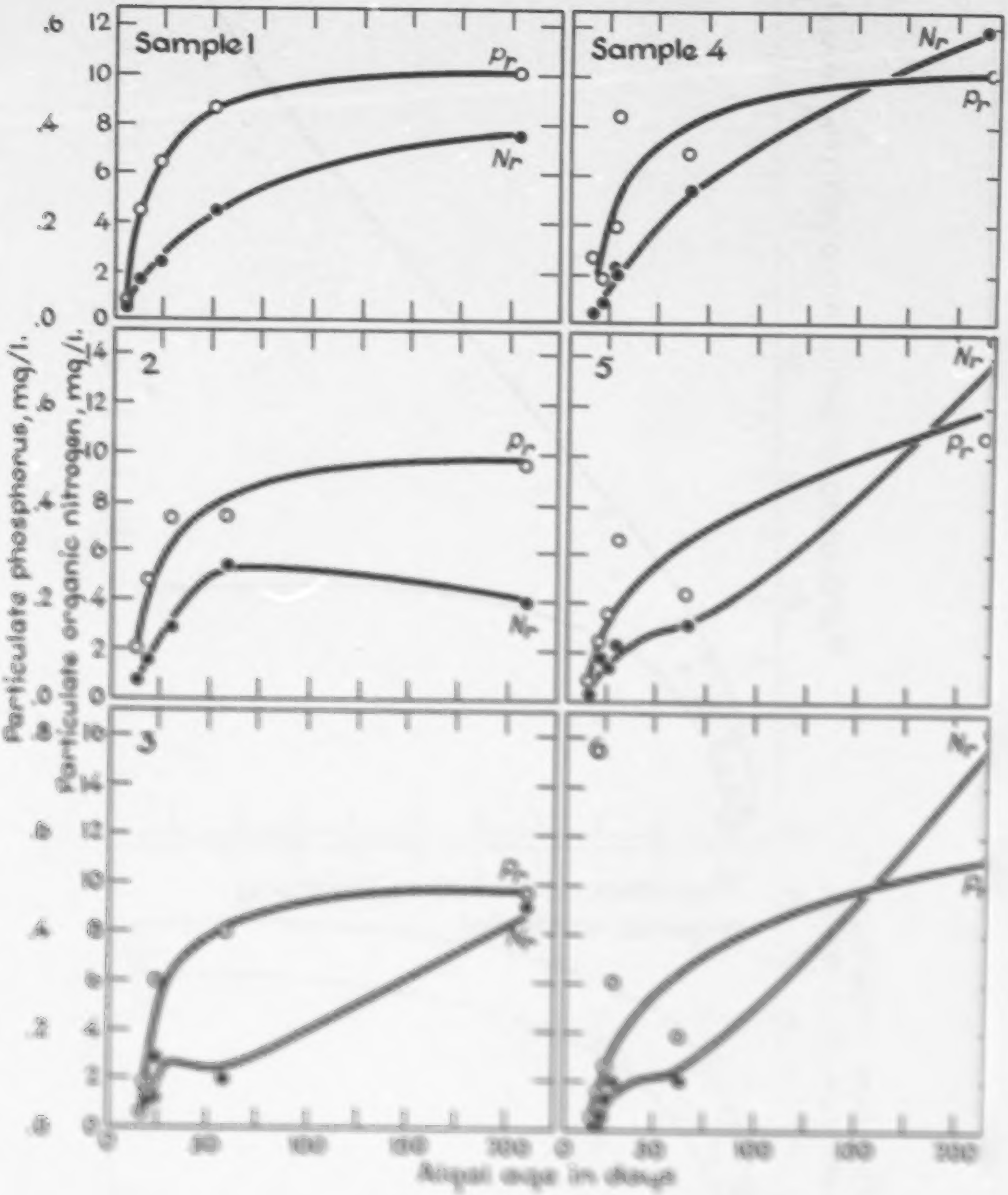

FIGURE 34. Quantity of organic nitrogen and phosphorus held in refractory organic material of algal systems.

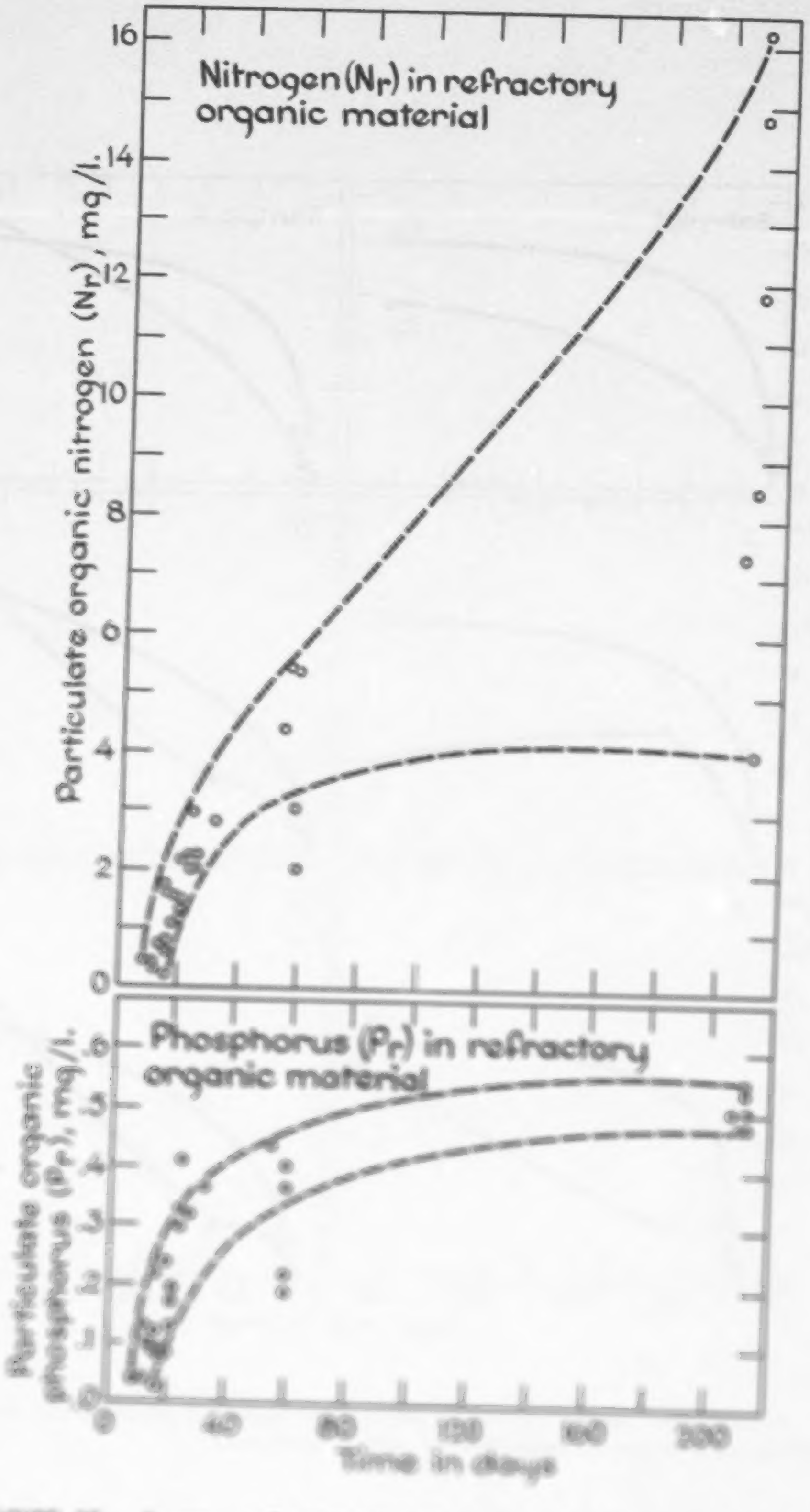

FIGURE 35. Summary of organic nitrogen and phosphorus held in refractory organic material of algal systems.

regeneration also decreased with increase in age of the algal systems.

Table 24. Summary of some of the Phosphorus* and Nitrogen+ Release Rates in Simulated Natural Algal Communities(Experiment III).

	NITROGEN RELEASE RATES					PHOSPHORUS RELEASE RATES		
	BRANCH: 1	2	3	4	5	1	2	4
ALGAL SYSTEM	AGE: (9-16 days)	(16-18 days)	(19-24 days)	(24-27 days)	(54-59 days)	(9-16 days)	(16-18 days)	(24-32 days)
1	6.2	5.1	---	1.9	0	476	26	2.2
2	3.7	1.7	1.8	0	0	68.6	24	2.8
3	39.2	20.8	21.6	6.3	4.5	424	252	49.4
4	64.2	74.5	2.0	0.9	1.4	417	74.6	19.3
5	12.1	3.7	0.4	2.8	0	422	34.6	31.2
6	23.0	10.1	12.4	7.4	4.1	778	111	78

*Nitrogen Release Rate X $10^{-4} = \dfrac{\text{mg/l N regenerated}}{\text{avg. mg/l SS - day}}$

*Phosphorus Release Rate X $10^{-6} = \dfrac{\text{mg/l P regenerated}}{\text{avg. mg/l SS - day}}$

EXPERIMENT IV

Young Algal Culture Studies

As noted in Experiment III the most surprising result of the simulated natural algal studies was the observation that the young algal cultures were often composed of a higher refractory organic fraction than older cells (see Table 19). Intuitively it appeared biodegradability should decrease with age. Examination of the young cells after decomposing in the dark for nearly a year indicated that most cells were still in good condition and were still a healthy green. In older branch samples where decomposition was more complete, the stabilized residue was brownish-yellow and no cells were recognizable. One difficulty with the study of young cells was that the concentration was too low (10 to 40 mg/l COD) to be sure of the accuracy of the results. For this reason further simulated natural studies were initiated. The last six natural waters shown in Table 14 were used in this experiment. Sample preparation and algal growth was carried out as in Experiment III. While these algal cultures were growing and nutrients were still in solution, approximately four liter samples were taken. Aeration was discontinued and the algae were allowed to settle for several hours in the light. The higher concentration of algae which resulted (greater than 85 mg/l COD in most cases) allowed more reliable analytical results. A 900 milliliter sample of the concentrated young algal cells was then placed in the decomposition vessels in the dark. In sample 8 approximately 20 large Daphnia were added to ascertain the effect of this animal on the decomposition process.

The chemical oxygen demand (COD) was the only variable measured in this experiment. The results are shown in Table 25. A shorter decomposition period was used in this experiment since data from the decomposition of young cells in Experiment III indicated that decomposition was nearly always complete within the first 50 days. Note that for the youngest cells, the refractory fraction exceeded 67 per cent. As the age of the algal cultures increased the refractory organic fraction fell to around 45 to 50 per cent. Thus further evidence was given for the occurrence of an extremely high refractory organic fraction in young algal cells.

Table 25. Refractory Organic Fraction of Young Concentrated Algal Cultures.

SAMPLE	AGE* (days)	DAYS UNDERGOING DECOMPOSITION	REFRACTORY FRACTION % of initial COD)
7-1	24	49	45
8-1	13	61	80
8-2 Contro	21	156	47
8-2	21	156	47
9-1	16	81	67
10-1	21	147	67
11-1	21	147	52
12-1	10	56	87
12-2	17	147	70

*Approximate days after initial "visible bloom"

EXPERIMENT V

Pure Culture Study

In order to support and further clarify the mechanisms which control decomposition, further experiments were designed using pure algal cultures. Because of the wide fluctuation of temperature in the natural environment, it was decided to determine the effect of temperature on algal decomposition and at the same time obtain information supporting the other phases of this study.

Pure cultures of Chlorella, Scenedesmus, and Chlamydomonas were grown on the "synthetic river water" (see Table 16, Experiment III), and a unialgal culture of Nitzschia closterium was grown on sterilized Monterey Bay sea water. Algae were grown at 20°C in the growth apparatus used in Experiment III. The apparatus was the same for this experiment except for added precautions to prevent bacterial contamination of the samples from the aeration mixture and other sources (as described in the procedure). The four above species were chosen in an attempt to obtain organisms with varying susceptibility to decomposition. Chlorella and Scenedesmus have been shown to be rather resistant to decomposition. Nitzschia closterium is representative of the diatoms, and the flagellate Chlamydomonas represents a more delicate phytoplankton form. Filamentous green algae and blue-greens were not included since it is felt that they would reflect decomposition falling between the limits as defined by the green algae and the flagellate. Note that the decomposition studies of branch samples 1 and 6 in the simulated natural algal cultures (Experiment III) were composed mostly of blue-green algae and so were all branches of sample 3.

Decomposition of both pure algal cultures and pure algal cultures after seeding were monitored at 3 to 4°, 20°, 25° and 35°C. All temperature environments were maintained in walk-in incubators except for 4°C for which a small refrigerator was used.

The seeded algal cultures had approximately 1 ml per liter of the following mixture added:

1. Mixed liquor suspended solids from an activated sludge pilot plant.
2. Algal pond bottom seed containing cladocerans and small crustacea.
3. Samples from acclimatized algal decay systems that had been fed various types of algae for a period of 6 months.

All analyses of carbon, nitrogen and phosphorus mentioned in the procedure section were used in this series of tests. Lipid analysis and Warburg studies were not used.

Figure 36 contains the results of the growth of pure cultures of *Chlorella*, *Scenedesmus*, *Nitzschia closterium* ("relatively pure"), and *Chlamydomonas*. Note the smooth appearance of these growth curves as opposed to growth exhibited by cultures under simulated natural conditions (Figures 12 to 17). Also note the similarity between the maximum yield of all the algal cultures except *Chlamydomonas*. At the time of the maximum yield for *Chlorella*, *Scenedesmus*, and *Nitzschia closterium*, the COD/N ratio was 96, 103, and 131, respectively. The reason that the COD/N ratio for an algal yield of 400 mg/l COD was not 80 for a system that had 5 mg/l N was because a certain fraction of the nitrogen was lost from the algal cells as the cells aged. As shown in Figure 36 the maximum algal yield did not occur for a variable time period of

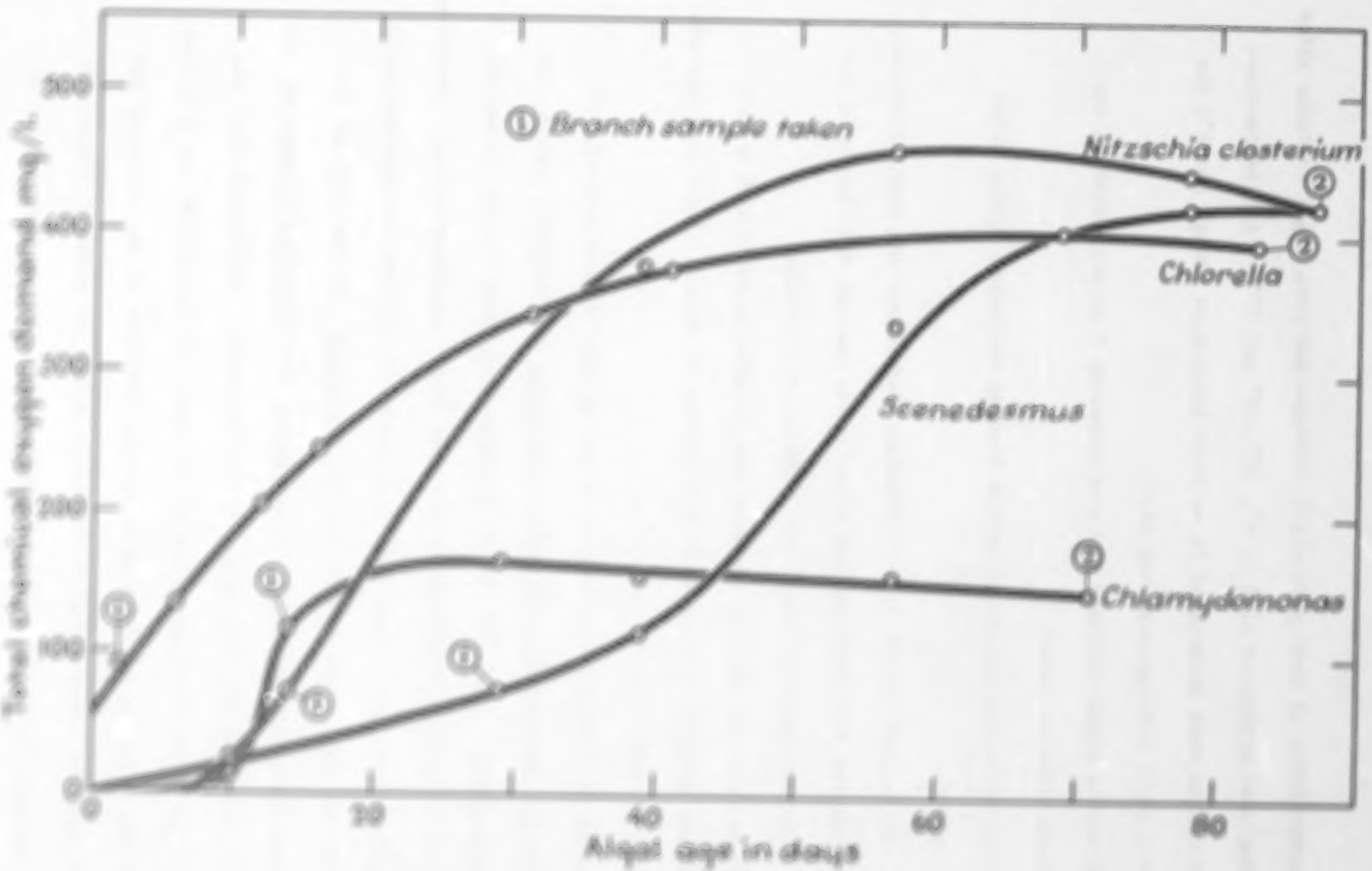

FIGURE 36. Growth of pure algae.

between 30 and 80 days. During this period *Chlorella* released the smallest quantity of nitrogen or approximately 16 per cent of the total in the system, whereas *Nitzschia closterium* released 38 per cent of the nitrogen back to solution. The maximum COD/N ratio with *Chlamydomonas* was only 66. Note the close correspondence of these maximum ratios to those observed with the simulated natural algal growth studies (Figures 18A to 18F).

In all of the experiments conducted in this study only *Chlamydomonas* was found to give off a large amount of its cellular material as dissolved organic material. In cultures of this algae approximately 50% of the cell material was found as dissolved organic material in the filtrate (using Whatman glass fiber filter, GF/C, 4.25 cm). A comparison of the production of extracellular material during growth by *Chlorella* and *Chlamydomonas* is shown in Figure 37. The COD represented by the soluble organic carbon seldom exceeded 10 per cent of the total COD with *Chlorella* whereas with *Chlamydomonas* it often approached 50 per cent (assuming 1 mg organic carbon equals approximately 2.5 mg COD(167)).

Duplicate pure algal samples were taken at the points designated by the circled points in Figure 36 and were placed in the dark at the various temperatures in a pure and seeded condition. In each case a sample corresponding to young algae (10 to 20 days old) and a sample corresponding to older algae (70 to 90 days old) was taken from each algal culture. In order to be able to directly compare the growth and decay of two of the pure cultures with the decay observed in Experiment III, *Chlorella* and *Chlamydomonas* were grown in the presents of bacteria and animals (1 ml activated sludge per liter growth media). Table 26

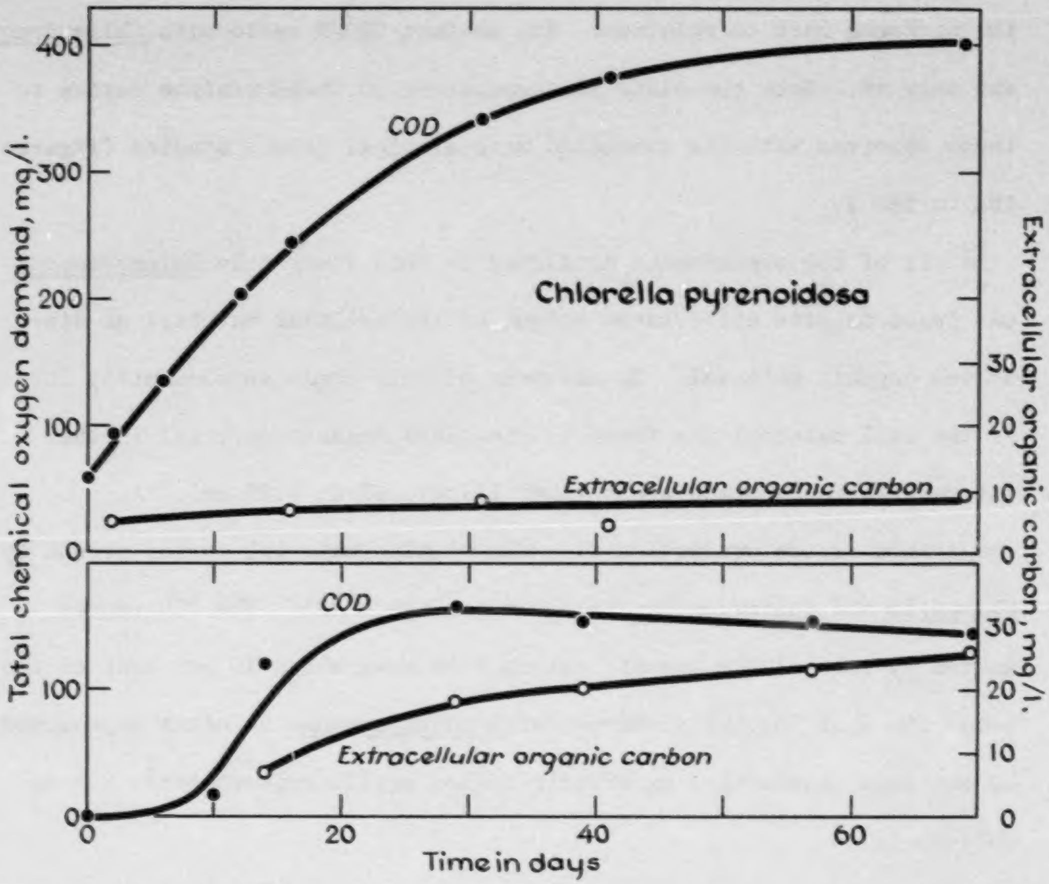

FIGURE 37. Correlation of production of extracellular organic carbon with algal growth.

includes a summary of the data of the algal system composition at the beginning of the decomposition study and at the end. Some of the nitrogen and phosphorus balances do not check in this table because of the addition of small amounts of nitrate-N and phosphate-P to the decomposer vessels with the addition of the seed after initial measurement. Since it was necessary to maintain some of the highly active microscopic animal populations in water with high concentrations of phosphate (used as buffer) and nitrates, it was difficult to avoid this addition. However, the determination of nitrogen and phosphorus balances were not of prime concern in this experiment. The decay of pure and seeded pure cultures may be represented by Figures 38 and 39. In all cases, except with Chlamydomonas (Figure 39), the decomposition of algae due to mechanisms within the algal cell alone was small.

With an acclimated bacterial seed and animal population, decomposition of all the algal cultures proceeded very rapidly. In most cases, however, the shape of the curve of decomposition followed a different pattern than that observed in the simulated natural decomposition studies.

Figures 38 and 39 also illustrate algal decomposition at four different temperatures after seeding with bacteria and animals. The amount of material respired even at 35°C in pure algal cultures was relatively small when compared to the effect of bacteria and animals on the rate and quantity of organic material removed at this temperature. Note that at 4°C the decomposition process was nearly halted in all cases. In the pure algal cultures a slight decrease in the organic material, as measured by the COD, was observed; whereas in the seeded culture a

Table 26. Algal System Composition Before and After Aerobic Decomposition, Experiment V.

	COMPOSITION AT START OF DECOMPOSITION														COMPOSITION AT END OF DECOMPOSITION												
SAMPLE	DECOM. TEMP. (°C)	ALGAL AGE (days)	COD, mg/l TOTAL	COD, mg/l SS	SS	FILTRATE ORG. C (mg/l)	NITROGEN mg/l as N TOTAL	SS	NH_3	NO_2	NO_3^-	PHOSPHORUS, mg/l P TOT.	TOT. SOL.	ORTHO	DAYS OF DECOMP.	COD, mg/l TOTAL	SS	SS	FIL. ORG. C (mg/l)	NITROGEN, mg/l as N TOTAL	SS	NH_3	NO_2^-	NO_3^-	PHOSPHORUS, mg/l as P TOTAL	TOTAL SOL.	ORTHO
Chlorella pure	4	10	92.5	80.5	70	5.0	5.30	5.20	0.15	0	0.15	.500	.040	0	95	61.0	60.2	48	3.4	4.60	3.00	0.25	0	0	.430	.025	0
	20	"	"	"	"	"	"	"	"	"	"	"	"	"	95	84.5	82.6	68	4.3	6.30	4.04	0.43	0	0.06	.550	.010	0
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	95	51.4	52.1	54	1.5	4.40	2.60	0.50	-	0.21	.460	.040	.025
Chlorella seeded	4	"	"	"	"	"	"	"	"	"	"	"	"	"	69	104	70.4	68	12.8	7.60	4.75	2.00	0	0.06	.593	.027	0
	20	"	"	"	"	"	"	"	"	"	"	"	"	"	69	43.0	32.8	52	2.4	2.00	2.40	0.07	-	2.90	.500	.113	.060
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	69	30.0	29.8	6	5.9	3.60	0.50	--	--	3.70	.500	.270	.222
"	35	16	244	230	154	6.4	7.20	5.33	1.2	0	0.05	.632	.008	0	79	34.4	31.0	30	4.9	2.35	1.20	0.35	0	2.50	.430	.245	---
"	4	93	395	360	222	5.5	6.60	4.20	1.1	0	0.06	.510	0	0	43	537	463	274	7.5	7.20	5.20	1.60	0	0	.820	.025	.015
"	20	"	"	"	"	"	"	"	"	"	"	"	"	"	43	177	157	118	4.5	6.30	5.44	0	--	0	.700	.015	.005
"	25	"	"	"	"	"	"	"	"	"	"	"	"	"	43	115	86.7	74	5.5	4.50	3.80	0.20	--	0	.600	.015	.015
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	43	64.1	52.4	46	3.5	2.70	2.00	0	--	0.01	.700	.200	.120
Chlorella grown with seed	4	15	83.4	74.7	64	2.5	5.20	2.32	2.50	0	0.03	.500	.015	0	109	77.0	77.3	72	1.6	4.60	3.16	0.60	--	0.08	.470	.010	.040
	20	"	"	"	"	"	"	"	"	"	"	"	"	"	109	23.4	23.0	30	2.5	3.20	0.64	2.00	--	2.15	.570	.340	.365
	25	"	"	"	"	"	"	"	"	"	"	"	"	"	109	26.7	24.6	32	1.4	2.50	0.92	0.70	0	2.15	.430	.350	.355
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	109	32.0	23.5	30	3.0	2.00	0.48	0.90	--	0.28	.430	.400	.133
Scenedesmus pure	20	29	73.5	--	62	6.8	5.20	2.76	1.20	0	2.60	.536	.020	0	45	54.6	48.2	40	8.0	4.30	2.40	0.70	0	1.60	.510	.010	0
	35	"	"	"	"	"	"	"	"	"	"	"	"	"	24	48.4	45.6	42	3.4	3.60	2.88	0.60	0	1.70	.530	.450	.425
Scenedesmus seeded	4	"	"	"	"	"	"	"	"	"	"	"	"	"	44	71.5	66.0	54	3.0	5.05	3.28	0.85	--	2.50	.710	0	0
	20	"	"	"	"	"	"	"	"	"	"	"	"	"	44	46.5	49.0	38	1.7	2.80	2.00	0.10	0	3.25	.770	.085	.065
"	25	"	"	"	"	"	"	"	"	"	"	"	"	"	44	17.5	17.5	16	1.3	0.80	0.62	0	0	3.50	.500	.450	.415
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	44	21.0	19.4	16	2.0	1.40	0.28	0	0	4.95	.620	.615	.475
"	4	87	420	390	260	7.7	5.90	2.88	1.50	0	0.08	.550	.030	.015	112	338	312	202	8.0	6.00	3.80	0.40	0	0.14	.530	.015	.050
"	20	"	"	"	"	"	"	"	"	"	"	"	"	"	112	88.5	84.5	62	9.5	4.00	1.88	1.80	-	2.10	.600	.265	.215
"	25	"	"	"	"	"	"	"	"	"	"	"	"	"	112	84.5	81.1	58	4.5	4.10	2.00	3.20	-	2.30	.580	.400	.500
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	112	76.4	68.7	52	4.0	2.70	1.36	0	-	2.70	.670	.415	.390
Chlamydomonas pure	20	5	119	100	74	6.4	7.80	5.13	1.6	0	0	.592	.040	.028	45	54.8	27.5	30	8.4	3.70	1.12	2.54	-	0.14	.480	.313	.147
	35	"	"	"	"	"	"	"	"	"	"	"	"	"	45	66.2	36.0	38	8.0	4.10	2.20	0.18	-	0.22	.520	.300	.167
Chlamydomonas seeded	4	"	"	"	"	"	"	"	"	"	"	"	"	"	45	72.1	54.7	54	19.8	5.80	2.60	3.06	-	0.14	1.030	.400	.313
	20	"	"	"	"	"	"	"	"	"	"	"	"	"	69	20.0	18.8	15	10.5	1.90	0.68	0.60	0	2.60	.780	.525	.475
"	25	"	"	"	"	"	"	"	"	"	"	"	"	"	69	19.0	11.7	19	4.5	1.00	0.47	0.25	-	3.85	.520	.385	.270
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	69	18.7	16.8	16	7.0	1.80	0.67	0.20	-	4.60	.680	.420	.450
Chlamydomonas seeded	4	62	146	82.2	76	26.6	5.90	2.68	2.1	0	0.10	.455	.087	0	106	83.0	38.0	55	22.5	5.60	1.20	2.60	-	0.68	.470	.285	.185
	20	"	"	"	"	"	"	"	"	"	"	"	"	"	106	34.9	16.7	30	9.5	2.70	1.00	1.00	-	2.74	.520	.365	.300
"	25	"	"	"	"	"	"	"	"	"	"	"	"	"	106	17.2	10.0	20	7.4	2.80	0.70	0.85	-	2.10	.570	.330	.155
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	106	31.1	20.0	26	6.0	2.00	0.28	0.70	-	2.65	.540	.330	.335
Chlamydomonas grown with seed	4	15	150	120	102	7.1	5.60	3.72	1.0	0	0.08	1.27	.267	.020	84	117	76.0	58	12.0	4.80	3.64	0.45	0	0	1.050	.050	0
	20	"	"	"	"	"	"	"	"	"	"	"	"	"	84	34.9	18.0	18	5.5	1.20	0.52	0	0	1.18	1.000	.722	.615
	25	"	"	"	"	"	"	"	"	"	"	"	"	"	84	33.1	10.0	14	5.0	1.00	0.68	0	0	1.26	1.020	.750	.625
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	84	35.0	20.2	14	5.0	1.30	0.64	0	0	1.00	1.000	.910	.825
Nitzschia closterium seeded	4	7	--	77.4	74	5.2	---	3.68	---	---	0.09	.920	.080	.012	96	--	77.5	74	4.9	5.70	3.20	-	0	0.05	.950	.010	0
	20	"	"	"	"	"	"	"	"	"	"	"	"	"	96	--	25.8	34	4.5	5.20	0.92	-	0	0	.880	.490	.565
	35	"	"	"	"	"	"	"	"	"	"	"	"	"	96	--	22.6	28	2.0	1.40	0.98	-	0	3.10	.760	.450	.475
"	4	87	---	420	260	32.5	5.60	3.48	1.80	--	0.11	.800	.050	.010	86	--	166	150	-	6.50	2.68	-	0		1.097	.015	.050
"	20	"	"	"	"	"	"	"	"	"	"	"	"	"	86	--	119	142	-	6.70	2.68	-	0	0.18	1.097	.125	.035
"	25	"	"	"	"	"	"	"	"	"	"	"	"	"	86	--	86	126	-	6.50	2.16	-	0	0.08	1.000	.445	.290
"	35	"	"	"	"	"	"	"	"	"	"	"	"	"	86	--	175	204	-	6.90	3.08	-	0	0.08	1.000	.615	.265

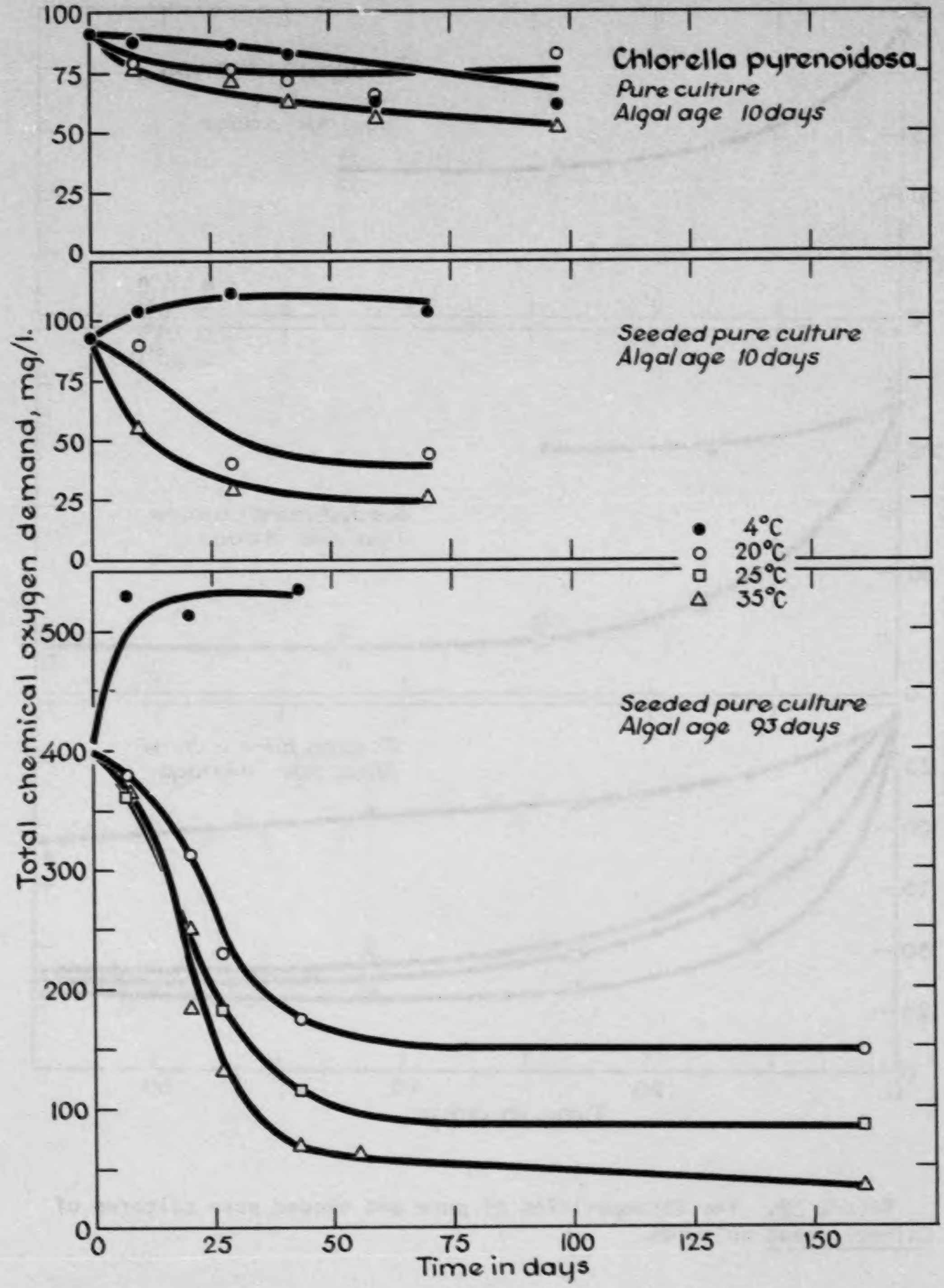

FIGURE 38. The decomposition of pure and seeded pure cultures of Chlorella pyrenoidosa.

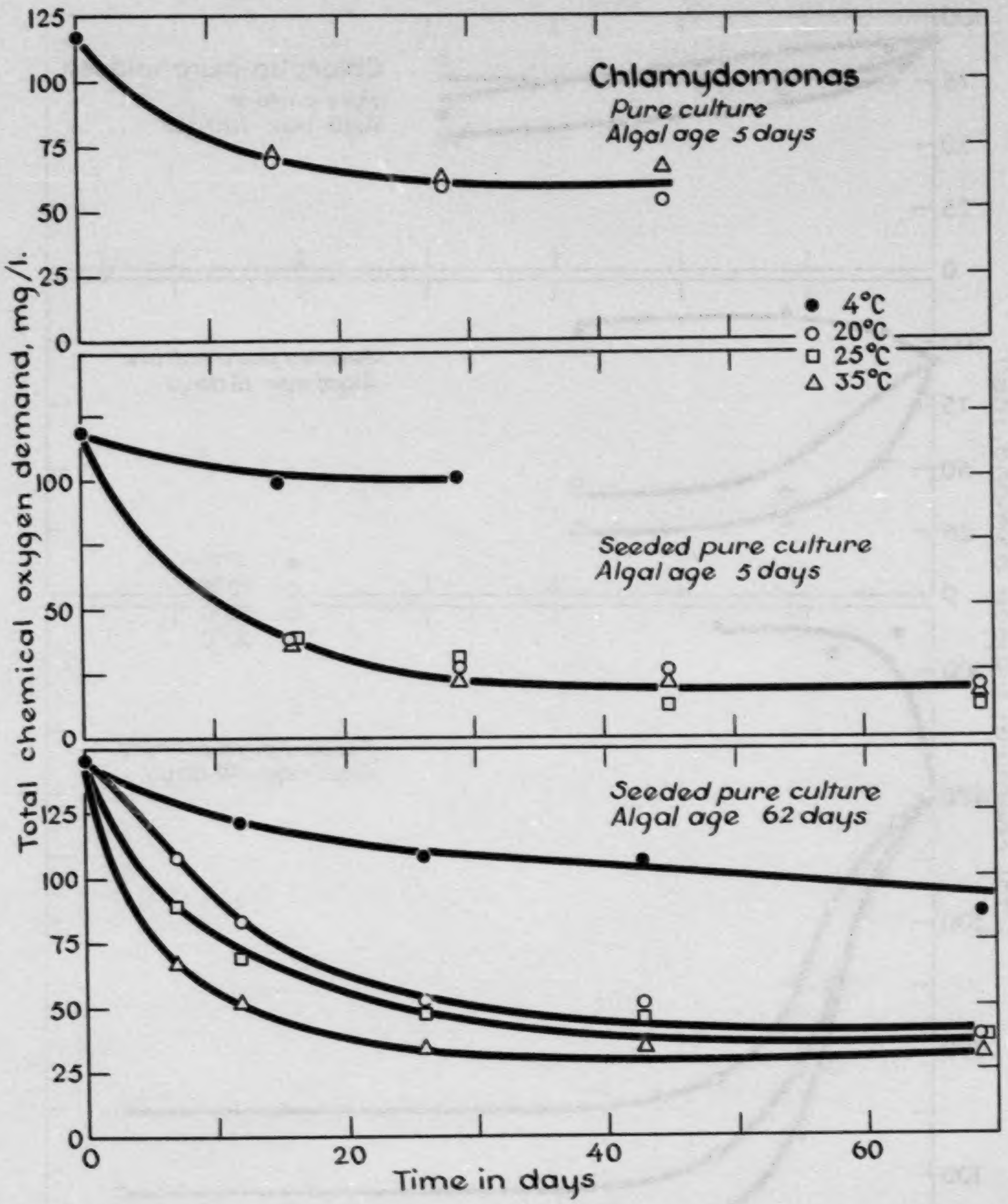

FIGURE 39. The decomposition of pure and seeded pure cultures of Chlamydomonas cultures.

significant increase was often observed. Apparently bacterial and algal cell synthesis (autotrophic) or the alteration of algal material and the inhibition of significant respiratory metabolism caused this increase of COD in the dark.

Table 27 contains a summary of the refractory fraction of seeded pure algal cultures. Decomposition appeared to be more complete than that obtained in the natural decomposition studies in some cases. In order to determine if this slight difference was due to the original presence of refractory organic material from bacteria and animals, new cultures of Chlorella and Chlamydomonas were grown in the presence of bacteria and animals; then placed in the dark to be decomposed. The results from this study (Table 27) indicated that bacteria and animals did not contribute a measurable amount of additional refractory organic material to the total organic material. Rather, the lower refractory fraction with pure algae probably resulted from a more suitable seed, higher temperatures and more delicate species (the flagellate). Note in Table 27 that with the young algal cultures of Chlorella, Scenedesmus, and Nitzschia closterium the refractory organic fraction measured at 20°C was 46.5, 63.3, and 33.4 per cent respectively. In most cases as the temperature increased to 25° and 35°C the refractory organic fraction decreased. With the delicate flagellate, Chlamydomonas, the refractory organic fraction was less than 20 per cent in some cases even at 20°C. Figure 40 summarizes this temperature effect on the refractory organic fraction. Since little decomposition occurred at 4°C the "apparent" refractory organic fraction was close to 100 per cent. At 20°, 25°, and 35°C the stabilization was completed and the refractory organic fraction decreased with increasing temperatures,

Table 27. Summary of the Refractory Fraction of Seeded Pure Algal Cultures.

SAMPLE	TEMP. (^{o}C)	ALGAL AGE (days)	PERIOD OF DECOMP. (days)	INITIAL COD (mg/l)	FINAL COD (mg/l)	REFRACTORY FRACTION (f)
Chlorella	20	10	70	92.5	43	46.5
"	35	10	70	92.5	30	32.5
"	35	16	79	244	34.4	14.1
"	20	93	43	395	177	44.8
"	25	93	43	395	115	29.1
"	35	93	43	395	64.1	16.2
Chlorella, grown with bacteria and animals	20	15	90	83.4	23.4	28.1
	25	15	90	83.4	26.7	32.0
	35	15	90	83.4	24	29.0
Scenedesmus	20	29	45	78.5	46.5	63.3
"	25	29	45	73.5	17.5	23.8
"	35	29	45	73.5	21.0	28.6
"	20	87	112	420	88.5	21.0
"	25	87	112	420	84.5	20.2
"	35	87	112	420	76.4	18.2
Chlamydomonas	20	5	69	119	20	16.8
"	25	5	69	119	19	16.0
"	35	5	69	119	18.7	15.7
"	20	62	106	146	34.9	23.8
"	25	62	106	146	17.2	11.8
"	35	62	106	146	311	21.3
Chlamydomonas, grown with bacteria and animals	20	15	49	150	34.9	23.3
	25	15	49	150	33.1	22.1
	35	15	49	150	35.0	23.3
Nitzschia closterium	20	7	69	77.4	25.8	33.4
	35	7	69	77.4	22.6	29.4
"	20	80	86	420	119	28.3
"	25	80	86	420	85.8	20.4
"	35	80	86	420	175	41.7

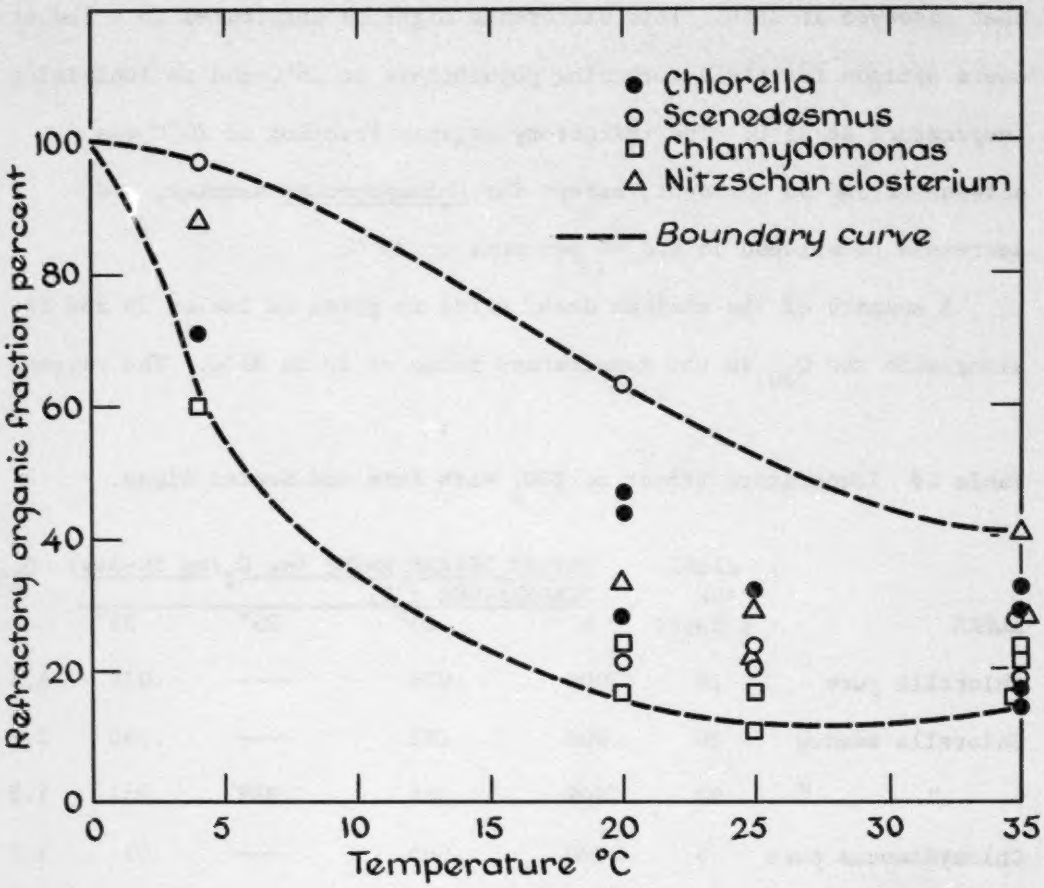

FIGURE 40. Effect of temperature on the refractory organic fraction of algal systems.

in general. At 25°C the refractory organic fraction appeared to be slightly lower than that found at 20°C, and in several cases the refractory fraction at 35°C was less than that at 25°C. However in some cases the apparent refractory organic fraction at 35°C was more than that observed at 25°C. This difference might be attributed to a temperature optimum for the decomposing populations at 25°C and an inhibiting temperature at 35°C. The refractory organic fraction at 20°C was between 22 and 50 per cent, except for Chlamydomonas samples, and decreased to between 18 and 40 per cent at 35°C.

A summary of the maximum decay rates is given in Tables 28 and 29 along with the Q_{10} in the temperature range of 20 to 35°C. The oxygen

Table 28 Temperature Effect on BOD_5 With Pure and Seeded Algae.

ALGAE	ALGAL AGE (days)	OXYGEN UPTAKE RATES (mg O_2/mg SS-day) TEMPERATURE (°C)				Q_{10}^*
		4°	20°	25°	35°	
Chlorella pure	10	.003	.026	----	.039	1.3
Chlorella seeded	10	.008	.102	----	.390	2.9
" "	93	.009	.028	.039	.051	1.5
Chlamydomonas pure	5	.020	.065	----	.094	1.3
Chlamydomonas seeded	5	.003	.082	.130	----	2.2
" "	62	.031	.104	.160	.140	2.1
" grown with seed	15	.017	.059	.069	.080	1.2
Scenedesmus, seeded	29	.010	.014	.070	.027	1.6
" "	87	.006	.018	.024	.036	1.7

* Q_{10} determined over temperature range 20 to 35°C

Table 29. Summary Maximum Dissolved Oxygen Uptake Rates (as determined from COD) for Pure and Seeded Pure Algal Cultures (Exp. V)

SAMPLE	ALGAL AGE (days)	TEMP. (^oC)	OXYGEN UPTAKE RATE $\frac{\text{mg } O_2}{\text{liter-day}}$	$\frac{\text{mg } O_2}{\text{mg SS-day}}$	Q_{10} (Determined over temp. range 20^o-35^oC)
Chlorella, pure	10	4	0.34	.006	
"	10	20	1.37	.022	1.1
"	10	35	1.49	.025	
Chlorella, seeded	10	4	0	0	
"	10	20	2.38	.063	
"	10	35	4.70	.077	
"	16	35	10.1	.090	
"	93	4	0	0	
"	93	20	4.15	.021	3.0
"	93	25	2.3	.012	
"	93	35	13.6	.085	
Chlorella grown	15	4	0.07	.001	
with seed	15	20	2.29	.046	1.5
"	15	25	2.57	.048	
"	15	35	4.34	.079	
Scenedesmus	29	20	0.69	.013	1.4
"	29	35	1.06	.020	
Scenedesmus, seeded	29	4	0.04	.001	
"	29	20	0.09	.015	1.9
"	29	25	2.27	.057	
"	29	35	1.88	.038	
"	87	4	0.74	.003	
"	87	20	4.45	.020	2.1
"	87	25	4.00	.018	
"	87	35	9.67	.053	
Chlamydomonas, pure	5	20	4.53	.080	1.0
"	5	35	4.66	.082	
Chlamydomonas,	5	4	1.04	.016	
seeded	5	20	5.33	.111	0
"	5	25	5.40	.110	
"	5	35	5.97	.100	
"	62	4	2.00	.029	
"	62	20	5.43	.092	1.9
"	62	25	8.15	.154	
"	62	35	11.2	.220	
Chamydomonas, grown	15	4	1.72	.019	
with seed	15	20	6.10	.088	1.6
"	15	25	6.90	.101	
"	15	35	8.35	.137	
Nitzschia closterium	14	4	0	—	
seeded	14	20	2.14	—	
"	14	35	3.18	—	
"	87	4	3.74	—	
"	87	20	2.14	—	
"	87	35	4.64	—	

uptake rates presented in Table 29 were estimated to equal the change in COD as determined over the steepest part of the decay curve, divided by the average suspended solids, multiplied by the period of time over which the change in COD was measured. The decomposition rates in Table 28 were obtained from BOD_5 tests run when the algal samples were initially placed in the dark at various temperatures. The oxygen usage as determined from the COD change measures only the carbonaceous demand whereas the BOD test measures this demand plus the demand from nitrification and other sources not directly related to the oxidation of organic carbon. In the absence of nitrification the change in COD will be approximately equal to the BOD_5. Although a fair correlation exists between the rates given in Tables 28 and 29 a good relation should not necessarily be expected since the time period over which the change in COD was measured was between 3 to 10 times longer than the time period used in BOD_5 rate determinations given in Table 29 . The change in COD and the oxygen utilized (as determined by using algae from Experiment III, and the Warburg analysis) were found to check within 10 per cent in several experiments in this study. Because of the dependence of the rate of algal decomposition on the seed, and acclimation of both the seed and the algae to the test temperature, these temperature effect values are not truly definitive, but only indicate the possible effect temperature can have on the decomposition rate.

A few of the rates of decomposition of pure algae induced by the acclimated bacteria and animals were considerably higher than the observed natural decomposition rates observed in Experiment III. Considering the amount and type of seed material involved in these studies it is felt that algal decomposition rates in nature will seldom exceed those reported in this study. Thus the decomposition rates observed in the simulated natural decay study (Experiment III) are believed to represent "average" or expected decay rates, whereas these results with seeded pure algal cultures are believed to represent the maximum rates of decomposition that can be expected. In the simulated natural study the decomposer seed consisted of all bacteria which were originally present in the sample. However, in the seeded pure cultures the bacteria and animals added were highly adapted to the decomposition of algae since they were taken from processes such as aerobic algal pond bottoms. Also in the simulated natural study it was noted that there was little buildup of soluble organics. This was probably because the decomposer activity assimilated any algal products which became available to them as the algae grew in the light. However in the pure culture studies, there was a noticeable buildup of soluble material in some cultures in the light. It can also be assumed that many of the cells lost their resistance to decomposition as they aged. Thus when an appropriate seed was added to the pure algal material it would be expected that high decomposition rates might occur. Unless the algal cells are killed by some environmental factor such as pollution it may be concluded that the rates in this experiment will seldom be equaled under natural circumstances.

Nitrogen and Phosphorus Regeneration in Pure and Seeded Algal Systems

The mechanisms that control nutrient regeneration are clearly indicated in the results from the pure culture studies. In several samples immediately after the pure cultures were placed in the dark a small amount of ortho phosphorus appeared in solution (0.01 to 0.1 mg/l P) only to be reabsorbed again. Figures 41 and 42 illustrate nutrient regeneration in pure cultures and in similar pure culture seeded with bacteria and animals. Table 30 summarizes the quantity of nitrogen and phosphorus regeneration in pure cultures. Nutrient regeneration by the algal cell itself is small in most instances except with *Chlamydomonas* which as in other aspects of decomposition is an exception. Thus in most cases the bacteria and microscopic animals mediate the release of a large amount of the nitrogen and

Table 30. Regeneration of Nitrogen and Phosphorus from Decomposition of Pure Cultures of Algae.

ALGAE	PERIOD OF DECOM. (days)	TEMP. (°C)	% COD DECREASE	N RELEASED (mg/l)	N RELEASED %	P RELEASED (mg/l)	P RELEASED %
Chlorella	95	4	34	1.60	34.8	.04	8
"	95	20	9	2.30	36.5	.01	2
"	95	35	45	1.80	41.0	.04	9
Scenedesmus	45	20	26	0.48	17.0	.01	2
"	24	35	34	0	0	.025	4
Chlamydomonas	45	20	54	4.01	78	.31	60
"	45	35	44	2.93	57	.30	60

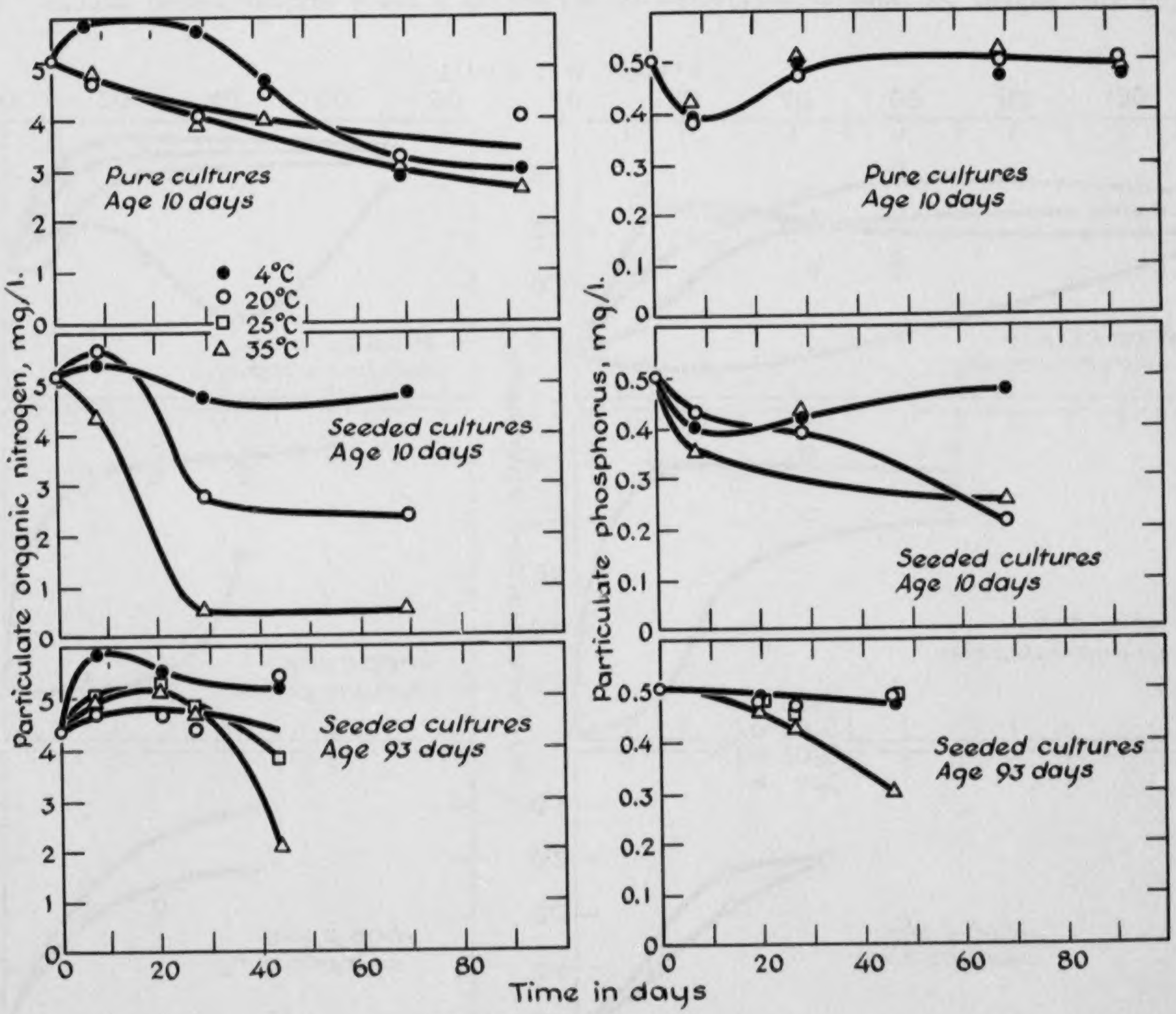

FIGURE 41. Change in particulate N and P during the decomposition of pure and seeded pure Chlorella pyrenoidosa cultures.

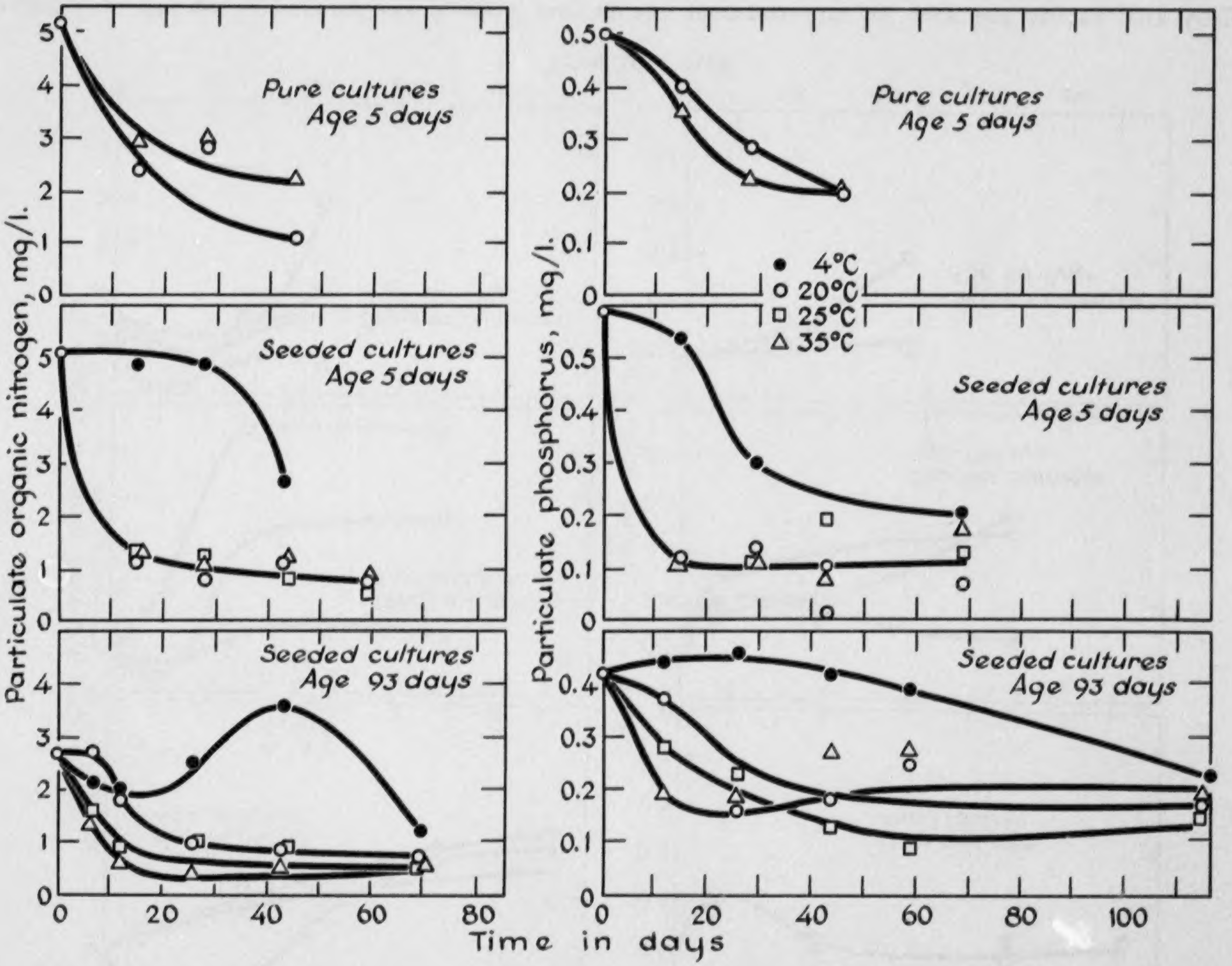

FIGURE 42. Change in particulate N and P during the decomposition of pure and seeded pure Chlamydomonas cultures.

phosphorus to solution. In most instances the regeneration of particulate nitrogen and phosphorus reflected the change in organic mass of the system (as measured by the COD).

Tables 31 and 32 summarize most of the information on the quantity and rates of nitrogen and phosphorus regenerated. As in Experiment III age was found to have a determining effect on the quantity of nutrient regeneration. In most cases with young Chlorella (10 to 16 days old) greater than 50 per cent of the nitrogen and phosphorus was regenerated. As the algae aged (95 days) the quantity of regeneration approached zero (see Table 31). Table 32 also shows that as the cultures aged the rate of regeneration decreased.

However, besides the aging effect there was also a temperature effect. This is clearly illustrated with Scenedesmus. At 20 degrees only 27 and 12 per cent of the nitrogen and phosphorus was released. At 25°C this increased to 78 and 71, respectively; and at 35°C 90 per cent of the nitrogen and 88 per cent of the phosphorus was regenerated. As noted in Table 32 regeneration at 4°C was often zero. Thus it can be seen that two variables - algal age and the decomposition temperature - largely control nitrogen and phosphorus regeneration.

The regeneration rates given in Table 32 for the seeded pure cultures can be compared to the regeneration rates in Table 24 given for the simulated natural study (Experiment III). Note that the rates are comparable but that some of those given in Table 32 at 25° and 35°C exceed those of Table 24 (for 20°C).

Table 31. Nutrient Regeneration With Seeded Pure Cultures.

SEEDED ALGAL CULTURE	TEMP. (°C)	ALGAL AGE (days)	PERIDD OF DECOMP. (days)	COD/N		%N			%P
				(Ini.)	(Final)	REGEN.	(Ini.)	(Final)	REGEN.
Chlorella	20	10	70	18.5	21.5	60	185	215	60
"	35	10	70	18.5	60	90	185	120	50
"	35	16	79	46	28.7	77	488	89	23
"	20	93	43	92	33.6	0	790	322	0
"	25	93	43	92	30.3	12	790	153	0
"	35	93	43	92	22.9	37	790	128	0
Chlorella, grown with seed	20	15	90	19.8	36.6	85	173	234	79
	25	15	90	19.8	29	78	173	178	69
	35	15	90	19.8	50	89	173	240	79
Scenedesmus	20	29	45	26.6	23.3	27	144	103	12
"	25	29	45	26.6	28.3	78	144	117	71
"	35	29	45	26.6	75	90	144	344	88
"	20	87	112	146	47	35	764	203	21
"	25	87	112	146	42	30	764	527	71
"	35	87	112	146	56	43	764	294	43
Chlamydomonas	20	5	69	23.2	29.5	87	23.8	30	87
	25	5	69	23.2	40.4	87	23.8	141	73
"	35	5	69	23.2	27.9	87	23.8	109	66
"	20	62	106	54.8	34.9	63	393	225	58
"	25	62	106	54.8	24.6	74	395	115	60
"	35	62	106	54.8	111	90	395	182	54
Chlamydomonas, grown with seed	20	15	49	40.3	67	86	150	124	72
	25	15	49	40.3	49.7	82	150	103	68
	35	15	49	40.3	54.7	83	150	167	79
Nitzschia closterium	20	7	69	20.9	28	71	84	82	66
	35	7	69	20.9	23	74	84	98	77
"	20	80	86	120	44.4	23	420	169	29
"	25	80	86	120	39.7	38	420	170	50
"	35	80	86	120	57	12	420	454	62
			Average		41.9			187	

Table 32. Summary of Some of the Phosphorus^{+} and Nitrogen* Release Rates in Pure and Seeded Algal systems.

ALGAL SYSTEM	ALGAL AGE	TEMP. (^{o}C)	P RELEASE RATE	Q_{10} (P RATES) (Temp. Range 20 to 35^{o}C)	N RELEASE RATE	Q_{10} (N RATE) (Temp. Range 20 to 35^{o}C)
Chlorella, pure	10	4	0		3.95	
	10	20	0		1.87	2.0
"	10	35	0		4.51	
Chlorella, seeded	10	4	0		0.9	
	10	20	61.8	2.6	7.6	2.6
"	10	35	212		25.0	
"	93	4	0		0	
"	93	20	0		0	
"	93	25	0		1.73	
"	93	35	34.8		16.6	
"	16	35	45.4		7.8	
Chlorella, grown with seed	15	4	0		2.5	
	15	20	360	1.6	14.8	neg.
	15	25	279		13.7	
"	15	35	664		8.3	
Scenedesmus, pure	29	20	0		16.4	
	29	35	0		11.0	
Scenedesmus, seeded	29	4	0		--	
	29	20	0		12.0	1.6
"	29	25	1020	neg.	48.3	
"	29	35	641		22.1	
"	87	4	0		0.4	
"	87	20	87	1.7	2.1	1.9
"	87	25	165		3.0	
"	87	35	173		3.0	
Chlamydomonas, pure	5	20	143	1.2	24.8	1.3
	5	35	178		35.0	
Chlamydomonas, seeded	5	4	218		11.6	
	5	20	583	1.0	62.8	-1.0
"	5	25	583		61.6	
"	5	35	546		57.4	
"	62	4	110		3.2	
"	62	20	471	neg.	26.0	1.3
"	62	25	480		12.7	
"	62	35	388		36.7	
Chlamydomonas, grown with seed	15	4	0		0.2	
	15	20	448	1.3	10.9	2.5
	15	25	217		19.1	
	15	35	634		36.3	

$$^{+}\text{Release rate X } 10^{-6} = \frac{\text{mg/l P Regenerated}}{\text{Avg. SS mg/l - day}}$$

$$^{*}\text{Release rate X } 10^{-4} = \frac{\text{mg/l N Regenerated}}{\text{Avg. SS mg/l - day}}$$

EXPERIMENT VI

Chlorophyll Study

The chlorophyll content of algal cells differentiates algal material from other organic matter in natural waters. For this reason chlorophyll is often measured and used as a correlating factor to primary production, biomass, and oxygen consumption by algae. A study to ascertain the validity of using chlorophyll as a measure of algal organic matter was initiated. The complete growth cycle of four different algal systems was monitored by the following tests: COD, BOD_5, and the chlorophyll content. The four algal systems consisted initially of Chlorella, Scenedesmus, Ulothrix, and Lyngbya. In order to restrict the algal growth and decay to one algal specie the experiment was initiated under pure conditions; however, sterile conditions were not maintained throughout the length of the study. The results of this study are shown in Figures 43 to 46.

Normal growth curves were observed in all cases but one, with an initial rapid growth period followed by a long stationary phase and then a declining phase. Ulothrix did not exhibit all stages of grwoth because of the growth of a blue-green algae some time after this green algae initially bloomed. (Note once again that the limiting algal yield occurred at a COD/N between 80 and 100). As can be seen in Figures 43 to 46 the chlorophyll concentration decreased rapidly in all cases and showed no correlation with COD. Results from the BOD_5 studies were interesting in that they explained the susceptibility of the algae to decomposition at different stages of the life cycle (all

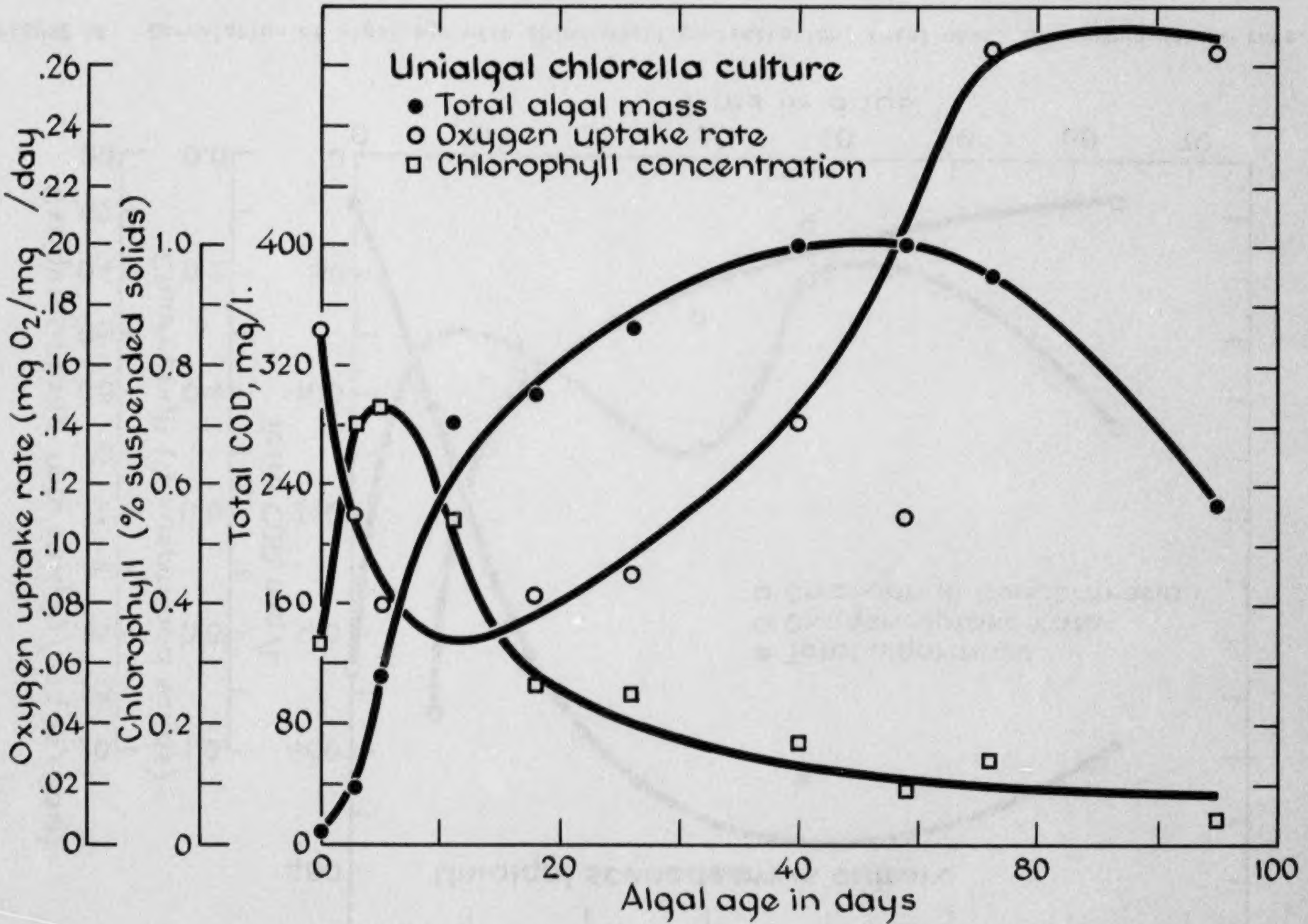

FIGURE 43. Correlation of algal age with chlorophyll concentration, total mass, and oxygen uptake rate.

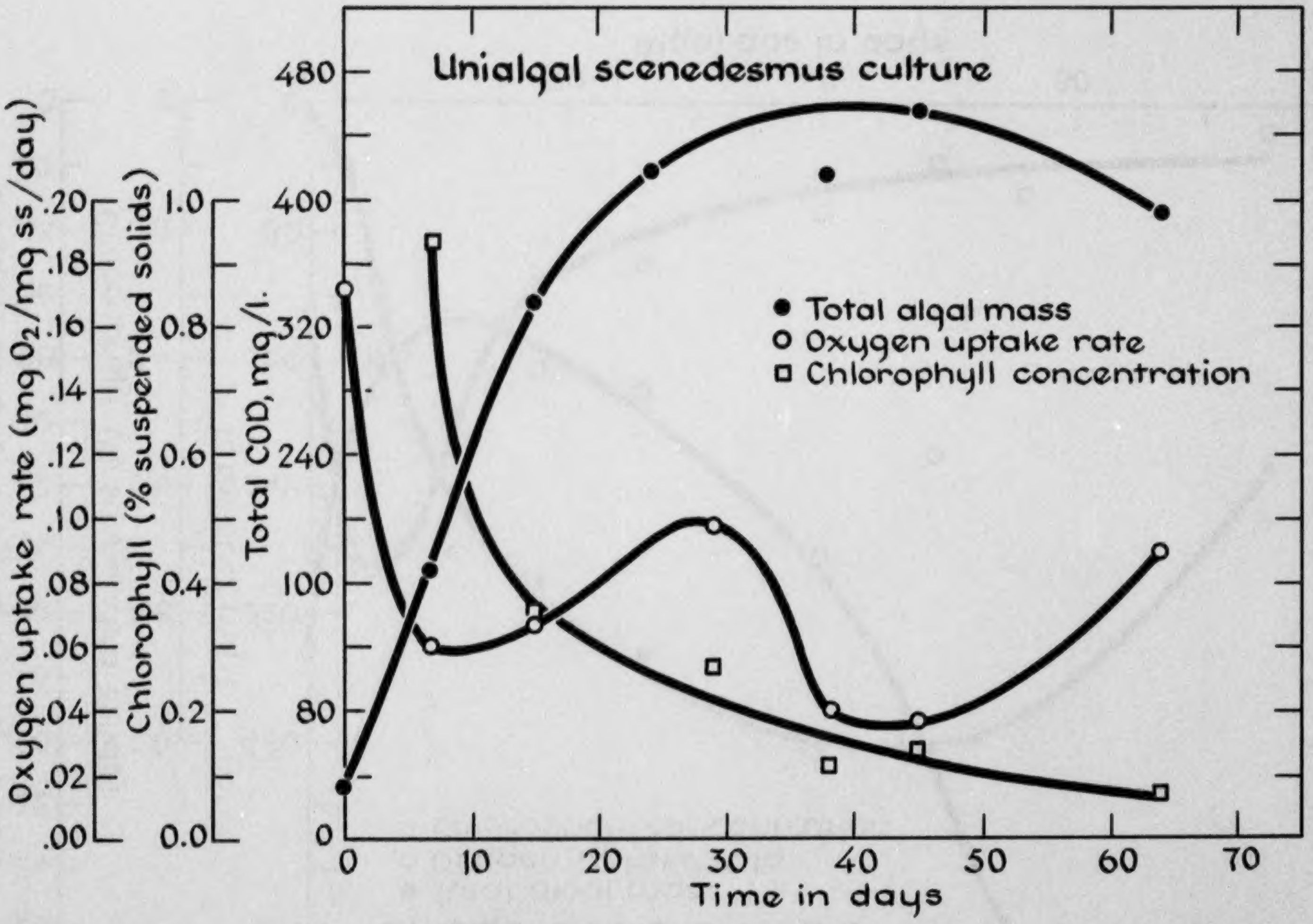

FIGURE 44. Correlation of algal age with chlorophyll concentration, total mass, and oxygen uptake rate.

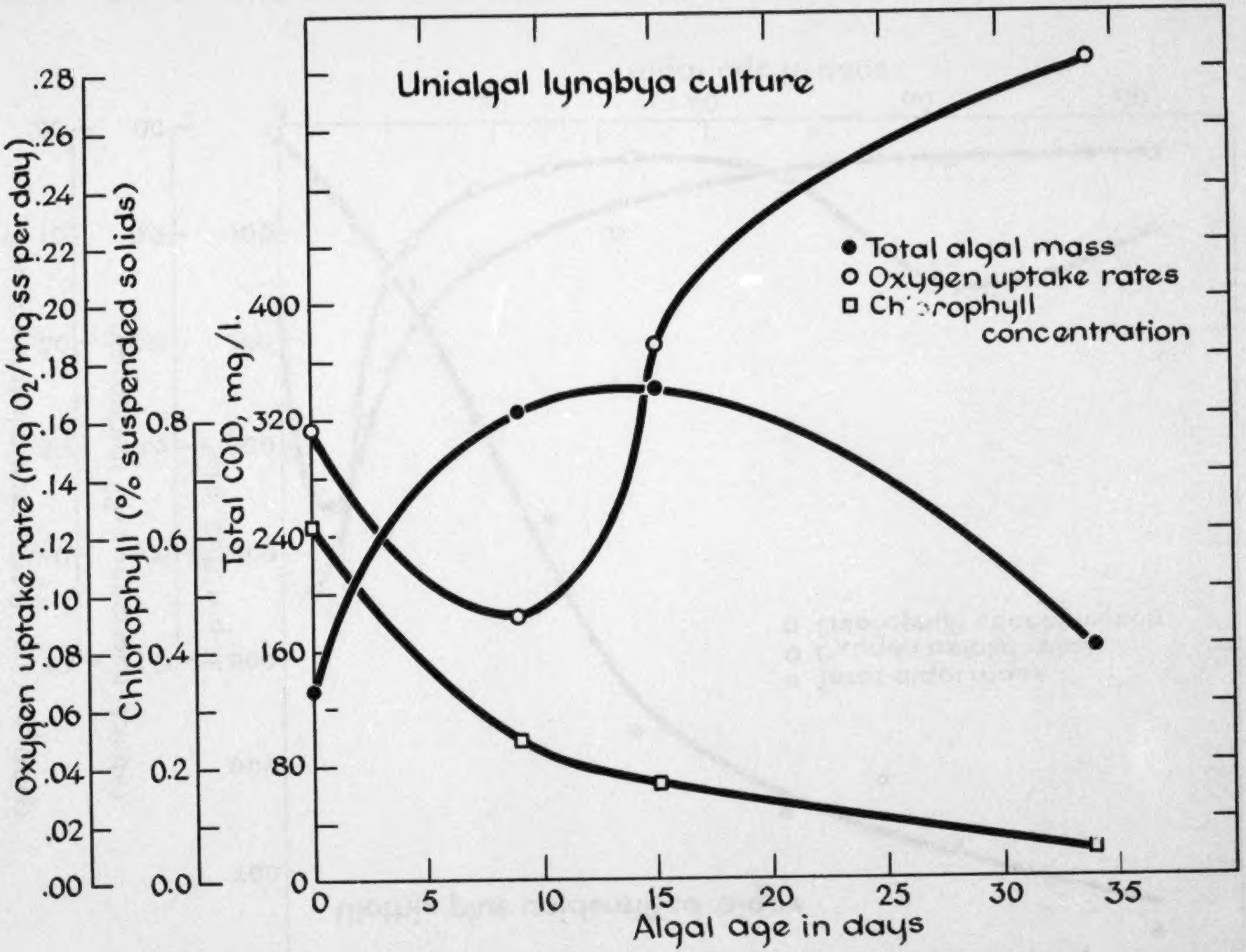

FIGURE 45. Correlation of algal age with chlorophyll concentration, total mass, and oxygen uptake rate.

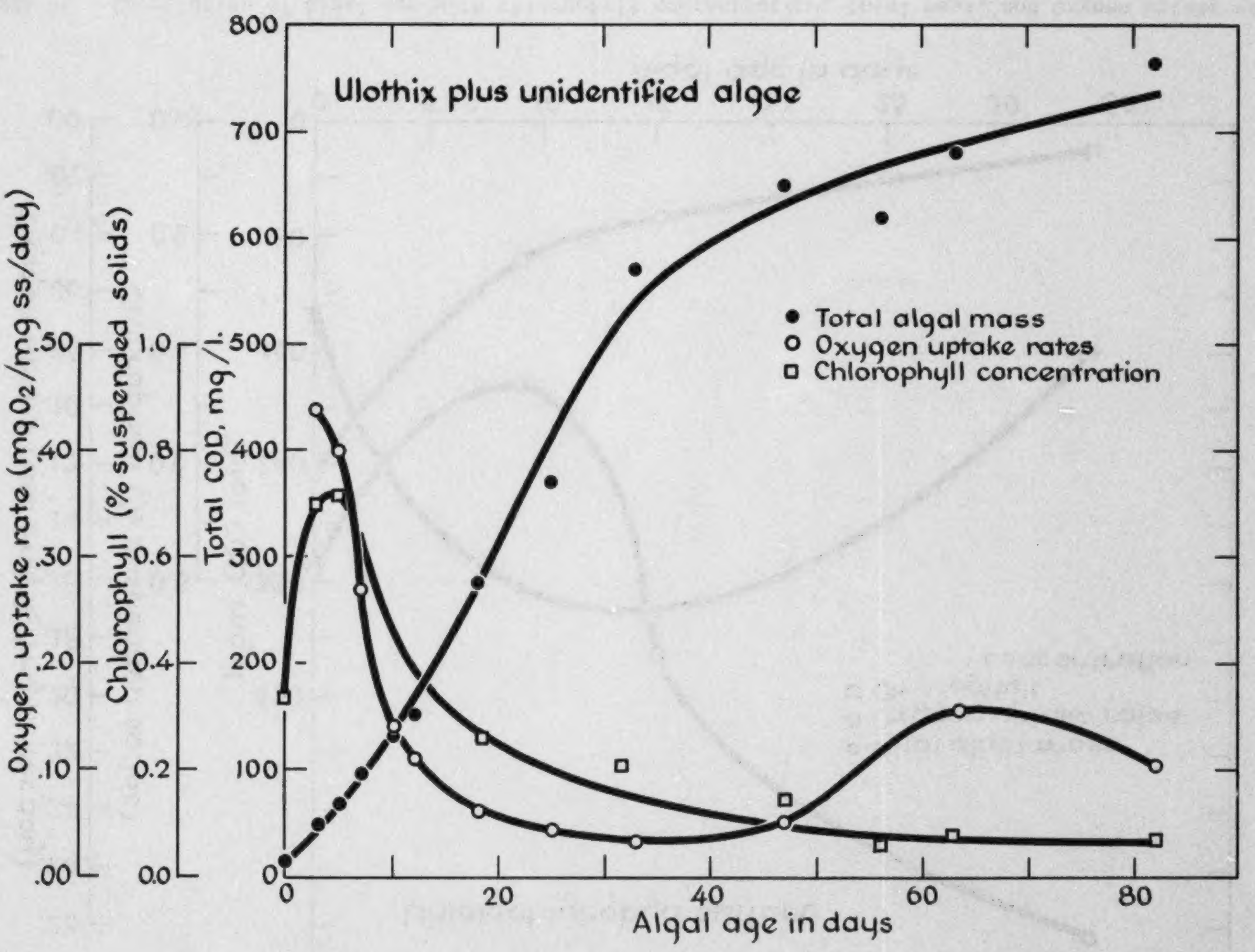

FIGURE 46. Correlation of algal age with chlorophyll concentration, total mass, and oxygen uptake rate.

BOD_5 analyses had the same seed added as in the decomposition studies in Experiment V). An unexpected minimum rate of oxygen demand was found in the early growth stages of all systems. The rate of oxygen demand then increased during the stationary and declining phase of all systems. Because of the bloom of a second species of algae with Ulothrix the stationary and declining growth phase did not occur in this system. Thus the results cannot be compared to the other three systems. The results of this experiment will be discussed further in the next section.

EXPERIMENT VII

Nutrient Supplementation Studies

Natural algae often develop in waters contaminated with other organic material. It is possible, if the algal decomposing populations are given a certain carbon source or available nutrient source, that the characteristics of algal decomposition and nutrient regeneration might be altered. To check these possibilities the following two studies were initiated. In one study decomposing algae were subjected to added organic material, and nitrogen and phosphorus; and in the other study the standard BOD_5 was compared to BOD_5 analyses using unsupplemented unbuffered dilution water.

In the first study seven different decomposition vessels as described in Experiment III were prepared using algae from sample 6 in Experiment III. The algae used had the following characteristics:

Age = 64 days, total COD = 227 mg/l, N concentration = 3.35% (COD basis), and P concentration = 0.22%.

To two 500 ml samples of the above algae, nitrate-N and phosphate-P were added to obtain a final soluble concentration of 12.5 mg/l N and 2.5 mg/l P in one and 37.5 mg/l N and 5.0 mg/l P in the other.

In order to simulate a case where algae are exposed to a very polluted condition, a large amount of easily degraded organic material was added in the form of Metrecal. Metrecal was added in one algal sample to obtain a Metrecal concentration of 1500 mg/l COD. A blank sample was also prepared with Metrecal in deionized water (Metrecal itself has sufficient nutrients and buffer for growth).

Finally two 500 ml algal samples had two animals added to them. One sample contained a Chinese algae eating tropical fish (approximately 3 grams, wet weight) and the other contained a large snail (approximately 3 grams, wet weight).

One ml of settled domestic sewage was added to all six of the above samples as well as to a control algal blank. These samples were then placed in the dark and allowed to decompose (as in Experiment III). Table 33 contains the results as obtained after 20 days of decomposition. In most cases decomposition was not significantly different from the control.

Table 33. Effect of Added Nutrients, Organic Material, and Animals on 70 day old (sample 6) Nutrient Deficient Algae Over 20 Days of Decomposition.

	CHEMICAL OXYGEN DEMAND OF UNITS, mg/l						
	ALGAL CONTROL	12.5 mg/l N 2.5 mg/l P	37.5 mg/l N 5.0 mg/l P	ALGAE + METRECAL	METRECAL CONTROL	3 GM SNAIL	3 GM FISH
Initial	222	222	222	1700	1500	222	222
Final	135	136	131	326	232	131	136

In another study to evaluate the effect of added nutrients all the BOD tests in the chlorophyll study (Experiment VI) and in the temperature study (Experiment V) were run in duplicate except one sample contained dilution water similar to that given by Standard Methods (170) (except the nitrogen source was nitrate-N), and the other was diluted with deionized water. The results from this study are shown in Table 34 with the initial nitrogen and phosphorus concentrations of the algal

Table 34. Comparison of BOD_5 with Standard BOD Dilution Water* ( ) and Deionized Water used as dilution Water.

SEEDED ALGAE	ALGAL AGE (days)	BOD DIL.	N (% COD in algae)	P	TOTAL COD (mg/l)	TEMP. (°C)	BOD_5, mg/l DILUTION WATER	
							DEIONIZED	STANDARD
Chlorella	4	3:1	1.8	.21	280	20	8.4	9.3
"	10	3:1	1.6	.18	304	20	13.2	15.0
"	26	4:1	1.6	.16	344	20	21.2	17.4
"	40	6:1	1.2	.16	400	20	28.2	25.8
"	49	6:1	1.1	.15	403	20	25.2	24.0
"	56	6:1	1.1	.15	380	20	41.4	41.4
"	75	10:1	1.7	.20	227	20	29.0	31.0
"	4	0.67:1	5.4	.54	34	20	5.1	4.8
"	104	3:1	4.1	.56	77	4	3.9	4.5
"	104	3:1	2.8	.70	23	20	3.6	1.2
"	104	3:1	3.4	.61	27	25	3.0	1.8
"	104	3:1	1.5	.31	32	35	3.0	3.9
Ulothrix	18	3:1	1.8	.18	276	20	15.2	17.4
"	25	3:1	1.3	.14	371	20	15.6	16.2
"	33	4:1	1.5	.09	570	20	24.	27.
"	47	6:1	1.6	.08	650	20	42	39.6
"	63	15:1	1.7	.07	683	20	39	43.5
"	82	10:1	1.7	.07	766	20	47	37
"	3	0.67:1	2.0	.24	30	20	5.2	7.2
Scenedesmus	1	0.67:1	11.1	2.5	344	20	3.7	3.9
"	7	3:1	3.0	.3	171	20	6.6	7.2
"	15	4:1	1.5	.14	336	20	10.0	14.6
"	29	6:1	1.2	.12	414	20	24.0	11.7
"	38	6:1	1.3	.12	415	20	16.2	13.2
"	45	6:1	1.0	.11	465	20	16.8	16.8
"	64	10:1	1.2	.10	382	20	20	19.0
"	112	3:1	1.1	.16	338	4	14.4	15.0
"	112	3:1	2.1	.30	88	20	6.1	4.8
"	112	3:1	2.4	.20	84	25	3.3	2.1
"	112	3:1	1.8	1.8	76	35	5.4	3.3
Chlamydomonas	103	3:1	4.0	1.2	17	25	2.4	2.1
	103	3:1	0.9	0.70	31	35	4.5	0.3
Nitzschia closterium	86	3:1	1.7	0.50	160	4	10.8	10.5
	86	3:1	2.2	0.70	119	20	11.7	9.3
"	86	3:1	2.5	.70	85	25	13.2	12.3
"	86	3:1	1.8	.5	174	35	17.1	17.4
Lyngbya and Chlorella	1	8:1	3.8	.40	132	20	15.6	12.0
	9	6:1	1.4	.15	326	20	13.6	12.0
"	15	6:1	1.7	.15	340	20	30.0	30.6
"	34	6:1	2.0	.30	160	20	26.0	29.0

*Nitrate used in place of ammonium ion as the nitrogen source material. There does not appear to be a significant difference in BOD_5 whether nutrients were added or withheld. In a study of the effect of dilution water on t he BOD_5 of algae Allen et al. (25) also showed that there was little effect on the BOD of algae for a wide variety of conditions.

In another short study, algae from natural waters of Experiment IV, with ages varying between 116 and 167 days, and with the algal nitrogen and phosphorus concentration (per cent COD) varying from 1.5 to 2.4, and 0.05 to 0.31, respectively, were used to determine the effect of added nitrogen on the decomposition rate. Added nitrate-N (5 mg/l) seemed to have no effect on the rate of decomposition of the algae over a period of time varying in length from 8 to 20 days. It might also be recalled that added nitrogen and phosphorus had no effect on the refractory organic material found in branch sample 6 (composited) of Experiment III (see Figure 27).

These studies indicate that the algae themselves yield adequate nutrients to support the decomposers. Thus it appears that excess nutrients or organic matter have little or no direct effect on the decomposition of algae. Also, because the decomposition of algal material is so slow, a significant change in the rate of decomposition might not be observed in these short studies. Further research on the effect of nutrients on the decomposition of algae and the determination of the mechanisms of nitrogen and phosphorus removal from algal material is needed to provide further insight into the understanding of nutrient regeneration.

VI. DISCUSSION OF RESULTS

ALGAL GROWTH

Although the decomposition of algae is the main subject of this investigation, the growth of algae has received considerable attention since it determines the characteristics of algal decay. Information presented in the Background Section indicated that a large amount of data is available in the area of algal composition and growth but that this information has not been correlated with decomposition. The results of this study indicate that algal composition varies over a wider range than has previously been reported, and that general statements regarding the maximum algal yield and its relation to the "available nitrogen and phosphorus can be made.

Variation of Algal Composition

Table 35 summarizes the maximum COD/N and COD/P observed in the natural and pure algal cultures. In four of the six studies (Experiment III), little indication of cessation of fixation of carbon was observed after nearly two years of exposure to diurnal light. Continuing growth was stimulated by the fixation of nitrogen above the initial level of 5 mg/l N, while the total phosphorus concentration remained at approximately 0.5 mg/l P. After the first group of algae reached a maximum COD/N ratio of approximately 100 in the absence of nitrogen fixation, subsequent growth of organisms such as the blue-green algae took place with the accompanying fixation of nitrogen and a resulting decrease in

Table 35. Maximum variation of nitrogen and phosphorus in algal communities.*

SIMULATED NATURAL ALGAL GROWTH STUDIES (EXPERIMENT III)

WATER	MAXIMUM COD/N	MAXIMUM COD/P
San Pablo Bay	155	1650
Tidal Sacramento River	105	1550
Fresh Sacramento River	50	1350
Searsville Lake	120	2480
Activated Sludge Effluent	90	2970
Synthetic River Media	76	2400

PURE ALGAL CULTURE GROWTH STUDIES (EXPERIMENT V)

Chlorella	96	790
Scenedesmus	103	765
Nitzschia closterium	121	527
Chlamydomonas	68	465

[*] As determined from the graphs of COD/N and COD/P

the COD/N ratio. As more nitrogen was fixed the COD/N ratio remained constant while the COD/P ratio became larger. The maximum COD/N ratios ranged from 55 to 155 in the absence of nitrogen fixation, and from 30 to 50 with fixation. The phosphorus concentration was more variable and fixation of carbon was not halted with a COD/P ratio of 2500. The added N/P ratio in these experiments was 10/1 during the initial growth, and the ratio in the cells remained fairly constant at this value. After nitrogen fixation had started, however, a N/P ratio in the cellular material as high as 87 (weight basis) was observed.

The minimum concentration of nitrogen observed in algal organic material of approximately 1.4 per cent on a volatile solids basis (COD/N = 100) agrees with that found by others (see Table 7). However, algal systems with as high a COD/P ratio as was measured in this study have not been reported. This may be for two reasons. First, these growth studies were carried out for a longer period than others; and secondly, all the systems here contained heterogeneous populations of algae, bacteria, and animals.

Potential Yield of Algae

The growth studies provide some insight into several important questions regarding algal growth. The most fundamental question is whether algae can grow in most natural waters if nitrogen and phosphorus are supplies at adequate levels. The stimulation of massive algal blooms by 5 mg/l N and 0.5 mg/l P in all natural waters collected including sources such as oligotrophic Lake Tahoe and mountain springs, and fertile waters such as the San Francisco Bay, indicates that some algal species, either endemic or imported, can probably grow in most natural waters if not suppressed by toxic substances or other environmental variables.

The next step in defining algal growth is to determine the maximum yield that can be expected from a given level of nutrients. This is an important consideration and has received a great deal of attention. Two approaches have been used. First, numerous investigators have tried to estimate the potential production of algae in natural waters by collecting the water, adding or withholding nutrients and exposing the sample to varying lighting conditions for various lengths of time (2,3, 186,187). After a predetermined time period the amount of growth is measured and this is reported as the "algal growth potential" or "AGP" (3) of the water. The simulated natural growth experiments in this study represent tests of this type. The major limitation in this approach is vividly illustrated in that algal production did not cease after a period of up to 633 days in some of the six samples as shown in Figures 12 to 17. There are two reasons for this continued growth. First, there are numerous algal species present in a natural water sample with varying abilities to grow under nutrient deficient conditions. Secondly, the nitrogen and phosphorus are not completely stabilized in the first cycle of algal growth. A certain fraction of the nutrients is available for future growth either through algal or bacterial enzymatic action. The time required to reach the maximum yield of algae when the above two factors are operative is in the order of years, which is an unreasonable analysis time period.

The second proposed method of estimating the potential yield of algae is one which relates the composition of the algae to available nutrients (138,148,149). For example, if algae are estimated to have nitrogen equal to 10% of their dry weight, then 1 mg/l N in a natural water will support the growth of 10 mg/l of algae. This, of course, is true only when all other nutrients are available in sufficient quantity to support growth. A modification of this method of estimating the potential yield of algae in natural waters appears to be the easiest and most valid approach to answering this question.

There is ample evidence that the presence of trace elements need not be a major consideration in the question of algal growth in natural waters (1,91,137,139). The explosive blooms stimulated by addition of 5 mg/l N and 0.5 mg/l P to all natural water samples indicates that some species of algae was always present and able to grow on the available trace elements. Thus it could be concluded that nitrogen and phosphorus are the major controlling nutrients in most natural waters.

An examination of the carbonaceous material fixed by the natural algal systems (Experiment III) indicates several significant factors. In all cases shown in Figures 12 to 17, the maximum algal yield was nearly independent of the phosphorus concentration (in one case 0.034% P (COD) did not cause cessation of algal growth), whereas the growth always showed a strict dependence on the nitrogen concentration. When the initial nitrogen was absorbed from solution a nitrogen deficient situation occurred and the nitrogen content of the system dropped to nearly 1% (VSS basis) and in all cases growth showed some signs of being inhibited. However, this nitrogen deficient condition was quickly modified by the fixation of nitrogen, presumably by the blue-green algae, in all six cases. In two of the six cases the total nitrogen increased only 20 to 30%, whereas in the others the increase was greater than several hundred per cent over a period of 300 days. In Sample 3 little nitrogen was fixed until around day 205 when fixation started at a high rate and was accompanied by a high rate of production of carbonaceous organic material.

The consistency between yield and the nitrogen content of the cells has already been discussed in terms of the COD/N ratios. Under favorable growth conditions when the available nitrogen sources (N) in mg/l include nitrite, nitrate, ammonia, and organic nitrogen, the maximum yield of algal material (C,mg/l COD) may be estimated using Equation 2: $C = a(N)$, where "a" = 100. When atmospheric nitrogen becomes available for algal growth then the maximum yield may be predicted using "a" equal to 50.

The variability of "a" in each of these equations would depend a great deal on the situation and the type of algal system. However, the maximum range of values which could be expected are shown in Table 35. The maximum range of "a" when atmospheric nitrogen is not available would be 55 to 155, with most values between 76 and 120. When atmospheric nitrogen is available "a" may have a maximum range of 30 to 55 (see Table 17). In both situations a "favorable" environment for algal growth must include phosphorus at a minimum concentration of 0.034 per cent of the total system chemical oxygen demand.

Although this study provides empirically defined values for the "minimum requirement" (134) of algal systems for nitrogen and phosphorus, the answer to one important consideration is unknown. This relates to the availability of various forms of nitrogen and phosphorus in natural waters for algal growth. As pointed out by Fitzgerald (180), it is not possible at present to determine the fraction of the total nitrogen and phosphorus which will be available. However, the literature appears to indicate that all biologically regenerated soluble (both organic and inorganic) nitrogen and phosphorus forms may be transformed by some biochemical process into a form which is available for algal growth. Several researchers have shown that most phytoplankton (and aquatic plants) have induced enzymes (190,191,192,193,194,195,196) which enable them to use a wide variety of inorganic condensed phosphorus compounds (197,198,199,200,201,202), nearly all the phosphorus in domestic sewage (203), and nearly all the phosphorus present in seawater (104,205). Although some research has shown that all nitrogen and phosphorus which is regenerated by algae can be reused for algal growth (99) this area is still in need of research.

The final question which the results from the algal growth study helps to clarify is which nutrient is more critical in the limitation of algal growth. It has been shown that a deficiency of silica can cause a cessation of growth with diatoms growing in a lake (136), but whether cessation of natural algal growth is usually caused by nitrogen or phosphorus limitation is still subject to question. Riley (206,207) and others have completed some work which indicates that phosphorus may limit algal yield in areas such as the ocean. However, in this research under simulated natural conditions where several species of phytoplankton continuously competed for the necessary growth nutrients, nitrogen was required at a much higher concentration than was phosphorus. Algae were seldom observed to grow with a nitrogen content less than 1.4 per cent (volatile suspended solids basis) whereas growth took place in some instances until the phosphorus concentration decreased to 0.05 per cent. The significance of these numbers can be further illustrated by considering the maximum ratio of nitrogen to phosphorus in the algal material and the concentration of phosphorus in the natural environment. A N/P ratio as high as 87 was observed in this study. A value of 0.07 mg/l phosphorus has been reported as that concentration which exists in natural water unaffected by outside phosphorus sources and photosynthesis (5), or in other words, this is the commonly occurring natural phosphorus equilibrium concentration. If this is true then a phosphorus limited algal growth situation might not occur until the nitrogen concentration reached 87 x .07 or 6 mg/l nitrogen. Since the nitrogen in natural water bodies is seldom as high as this, nitrogen should be more often of significance in limiting algal yield than phosphorus.

The above discussion of limitations of algal yield by nitrogen and phosphorus has neglected a great many variables. In any natural environment there will be parameters such as illumination and temperature that will have as much control over the results as will the presence of nitrogen and phosphorus. This work further defines the limiting concentration of nitrogen and phosphorus but a fruitful area for future research would be to determine the effect of natural parameters on these growth limiting nutrients.

THE DECOMPOSITION OF ALGAE

The discussion of algal decomposition and nutrient regeneration will be divided into two general areas. First, the hypotheses discussed in the Theory Section will be compared with the laboratory results. This will show where the hypotheses are applicable, and what expected error may be involved with their use. Second, the factors which control algal decomposition and nutrient regeneration, and the relative effect of these factors under various conditions will be considered.

The process of algal decomposition or carbon regeneration was postulated to follow two separate kinetic equations depending on the manner in which the algae were exposed to the decomposing bacteria and animals (see Figure 9). The general case of algal decay occurs when algae develop in a relatively pure culture and are attacked by the decomposers some time after the algae appear. The kinetics of this general case were hypothesized to conform to the second order equation, repeated here for convenience:

$$M = \frac{(M_1 - f\ M_o)Q}{1 + Q} + f\ M_o \qquad \text{Eq (8)}$$

where $$Q = \frac{M_o - f\ M_1\ e^{-(M_1 - f\ M_o)\ K''t}}{M_1 - M_o}$$

M_o is the total COD measured at time zero, M_1 is M_o plus a constant to account for the effect of the initial mass of decomposers plus antecedent conditions, i.e., respiration, age, etc., K'' is the decay rate coefficient, f is the non-biodegradable fraction of the COD, and M is the total organic mass (mg/l COD) at any time (t, in days) after decomposition is initiated.

The second case of algal decomposition occurs when the decomposers develop simultaneously with the algal community. This is hypothesized as a limiting case of the above and can be approximated using the following first order equation:

$$M = (M_o - f\ M_o)\ e^{-K't} + f\ M_o \qquad \text{Eq (9)}$$

where K' is the decay rate coefficient and the other variables are as defined for Equation 8.

In both cases of algal decay it is indicated that if the refractory fraction (f) and the rate of decay (K) of an algal system can be predicted, the decomposition kinetics will be completely defined. From several studies the rates of decay of algae under various conditions have been reported, and others have measured the refractory fraction of algae, but no study has correlated these two factors.

All experiments where bacteria and animals were allowed to develop simultaneously with the algae can be considered to be examples of decay by Equation 9 (all Experiments but V). In Experiment V where algae were grown pure and then seeded with bacteria and animals at different times the decomposition may be described by Equation 8.

Second Order Decomposition Kinetics

The decomposition of pure algal cultures and seeded pure cultures were used to investigate this mechanism of algal decomposition (see Experiment V). Because of the difficulty of defining M_1 in Equation 8, a trial and error fitting of the postulated equation to the observed data was attempted. Calculated data and observed data for a representative experiment are shown in Figure 47. This type of kinetics applied to pure cultures of Chlorella, Scenedesmus, and Nitzschia closterium; but with Chlamydomonas (Figure 39) decomposition appeared to follow first order kinetics. Also as the temperature increased, the decay kinetics for all algal systems approached first order type curves.

Two conditions affect the nature of the antecedent conditions as indicated by the value of M_1 and thus cause a deviation from the second order type of curve. In the case of Chlamydomonas the antecedent conditions are different than with others. Figure 39, for example, shows that its endogenous respiration rate is much higher (see Figure 38). Also, because of the accumulation of a large amount of biodegradable substrate in solution with the algae, the decomposer mass probably increased very rapidly. Both the high initial endogenous respiration rate and the rapid increase in the decomposer mass would cause a high rate of decomposition during the early stages. With this high initial rate, the decay kinetics would approach a first order type relationship.

Thus it is shown that second order kinetics as proposed in Equation 8 can be used to describe carbon regeneration in specially defined instances. However, the same relatively pure community of algae could be decayed at entirely different rates depending on the initial amount and kind of seed to which the community was exposed. Although the dependence of any algal system decay on antecedent conditions has been shown, the value for M_1 is unpredictable. The kinetics of decomposition are extremely sensitive to M_1 as illustrated in Figure 48. The smaller the

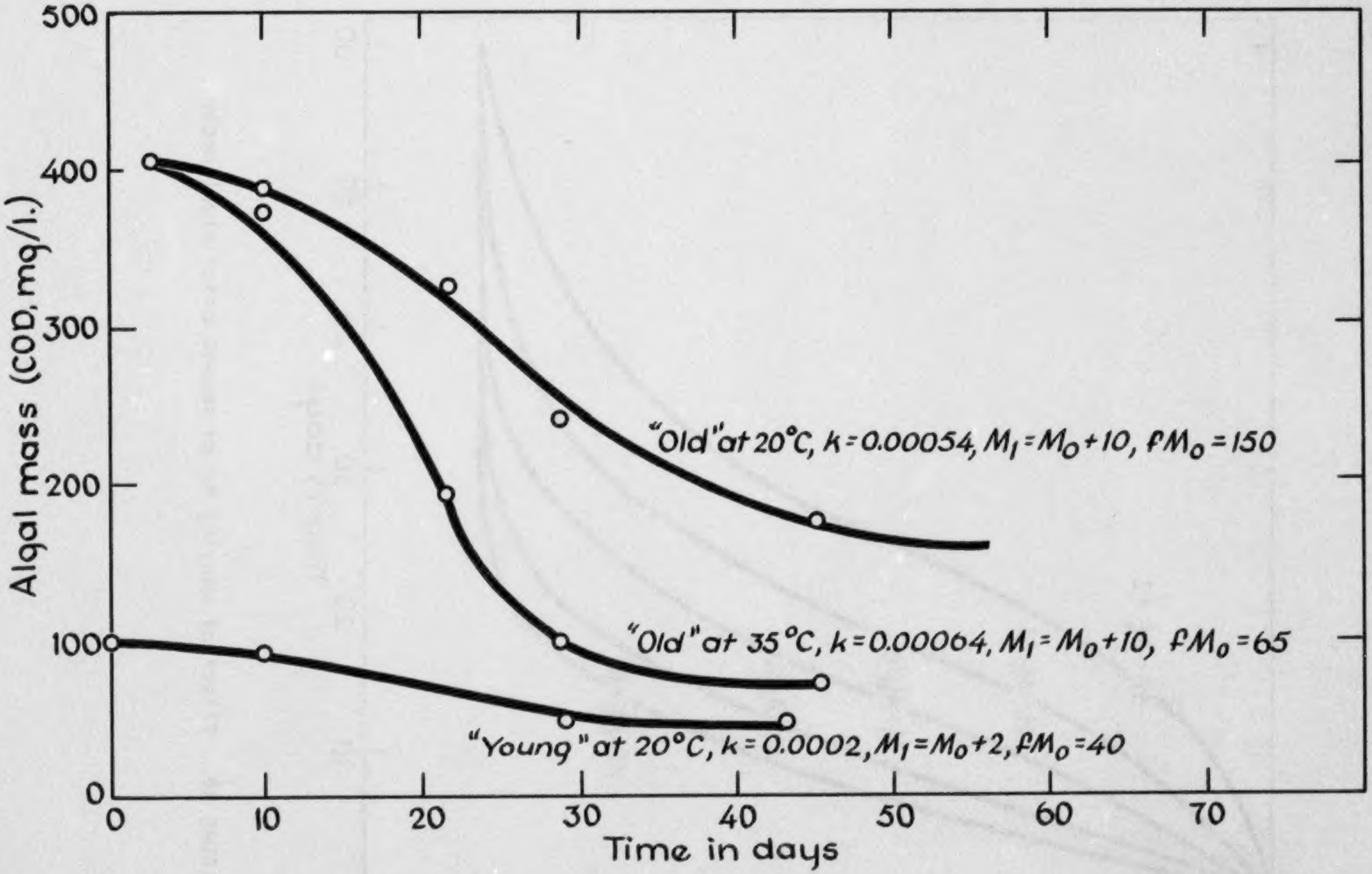

FIGURE 47. Application of second order equation to data for young and old seeded Chlorella cultures.

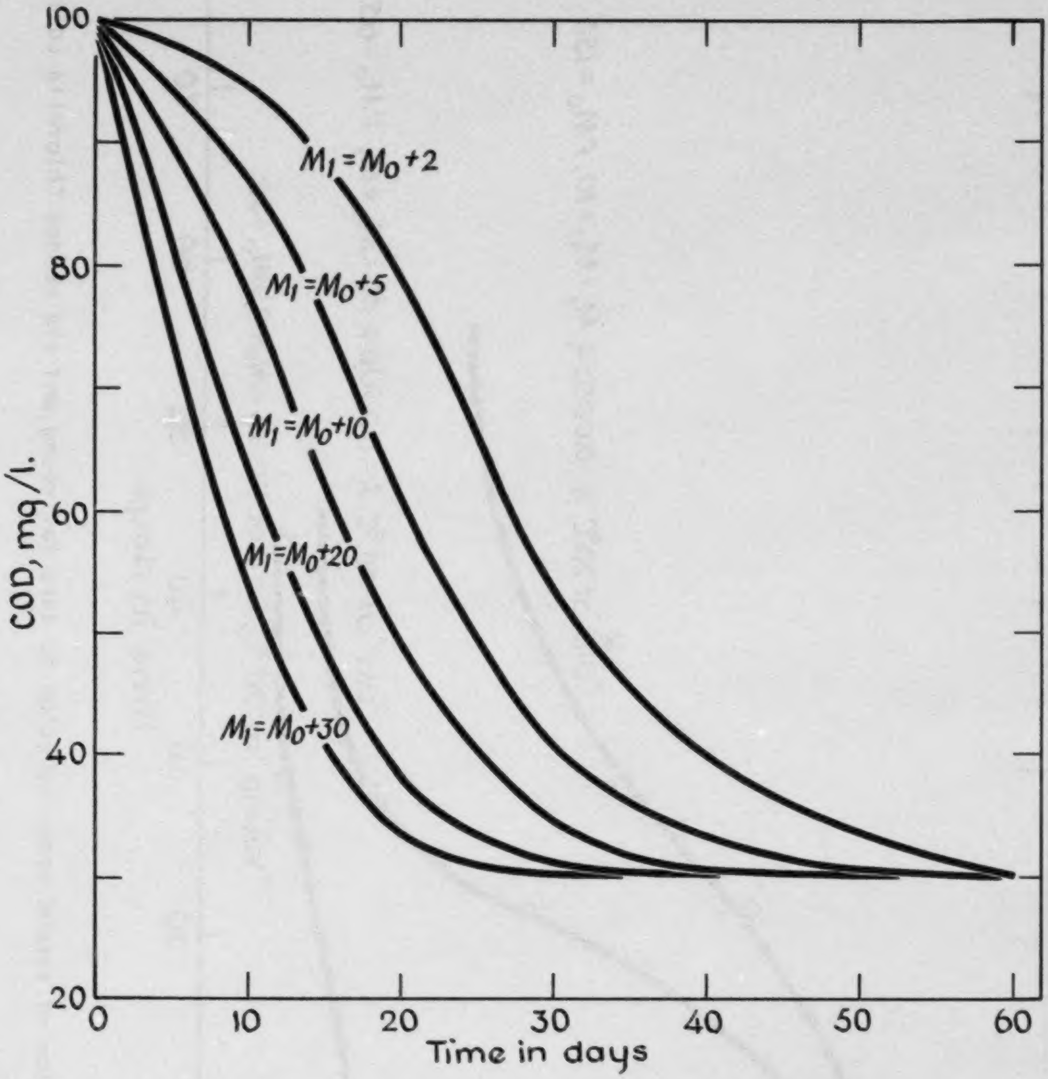

FIGURE 48. Effect of varying M_1 on second order algal decay.

difference between M_o and M_1, the more the shape of the curve reflects the common second order, or autocatalytic decay curve.

Figure 48 also illustrates the progression of the kinetics of decomposition from the general type described by a second order relationship to the boundary case described by first order kinetics. As M_1 becomes greater, the kinetics of decomposition approaches that which can be approximated by the first order equation.

Although second order decomposition has been shown to describe this type of decomposition, the difficulty in obtaining the decay rate coefficient and the decomposer mass decrease the value of this equation for predictive purposes. However, this is a rare type of decomposition and when it does occur it rapidly changes its characteristics to those approaching first order kinetics. It was found that when a first order curve was fitted to this data, the average error between all measured and calculated values $\left(\frac{\text{Calculated Value - measured value}}{\text{Calculated Value}} \times 100\right)$ was only 15.2 per cent with a standard deviation of 9.7 per cent. The largest error was found with young cells such as Chlorella. With cells such as Chlamydomonas, which exhibited a relatively high endogenous respiration rate in pure culture, the approximation with the first order relationship was much better. The average error between the calculated and measured value being only 5.6 per cent.

The above discussion indicates the usefulness of the second order equation together with a value M_1 which helps account for the effect of antecedent conditions. At the same time, these results also indicate the difficulty of applying the second order equation and indicate some justification for the use of the first order relationship to overcome this difficulty.

First Order Decomposition Kinetics

Algae which are present in most water bodies will tend to support a population of bacteria and animals with fluctuations produced by various environmental variables. With this continuous source of seed during algal growth, most natural algal decomposition should follow first order decay kinetics. The decomposition results from the simulated natural studies (Experiment III) were assumed to follow first order decay by Equation 9. All measured values of the organic mass were compared to the calculated value using the following procedure. The measured refractory fraction (f) of the initial total COD (see Table 19) was used in each case. By taking the natural logarithm of Equation 9, the following results:

$$\ln (M - f M_o) = K't + \ln (M_o - f M_o)$$

By plotting $\log_{10} (M - f M_o)$ versus time on semi-logarithmic paper and calculating the slope of the line of best fit (by eye), the first order decay rate coefficient can be obtained. This was done for 35 branch samples in Experiment III. The resulting decay rate coefficients (base 10) were multiplied by 2.3 to change to base "e" rates in order to make them directly applicable to Equation 9. These decay rate coefficients (K') are summarized in Table 36 and plotted against the age of the algal system in Figure 49. By applying the measured f and calculated

Table 36. Summary of Calculated First Order Decomposition Rate Coefficients (K') (from Experiment III).

ALGAL SYSTEM	BRANCH	ALGAL AGE (days)	DECOMOSITION RATE COEFFICIENT (K', day^{-1})
San Pablo Bay (1)	1	9	.100
	2	16	.034
	4	27	.019
	5	54	.010
	6	207	.011
Tidal Sacramento River (2)	1	14	.150
	2	19	.034
	4	33	.010
	5	59	.010
	6	212	.025
Fresh Sacramento River (3)	1	16	.140
	2	18	.052
	3	20	.040
	4	24	.067
	5	59	.014
	6	211	.021
Searsville Lake (4)	1	12	.080
	2	16	.034
	3	21	.054
	4	24	.032
	5	59	.014
	6	211	.021
Activated Sludge Effluent (5)	1	12	.028
	2	16	.041
	3	21	.026
	4	24	.021
	5	59	.030
	6	211	.015
Synthetic River Water (6)	1	16	.055
	2	18	.016
	3	21	.042
	4	24	.057
	5	59	.049
	6	211	.011

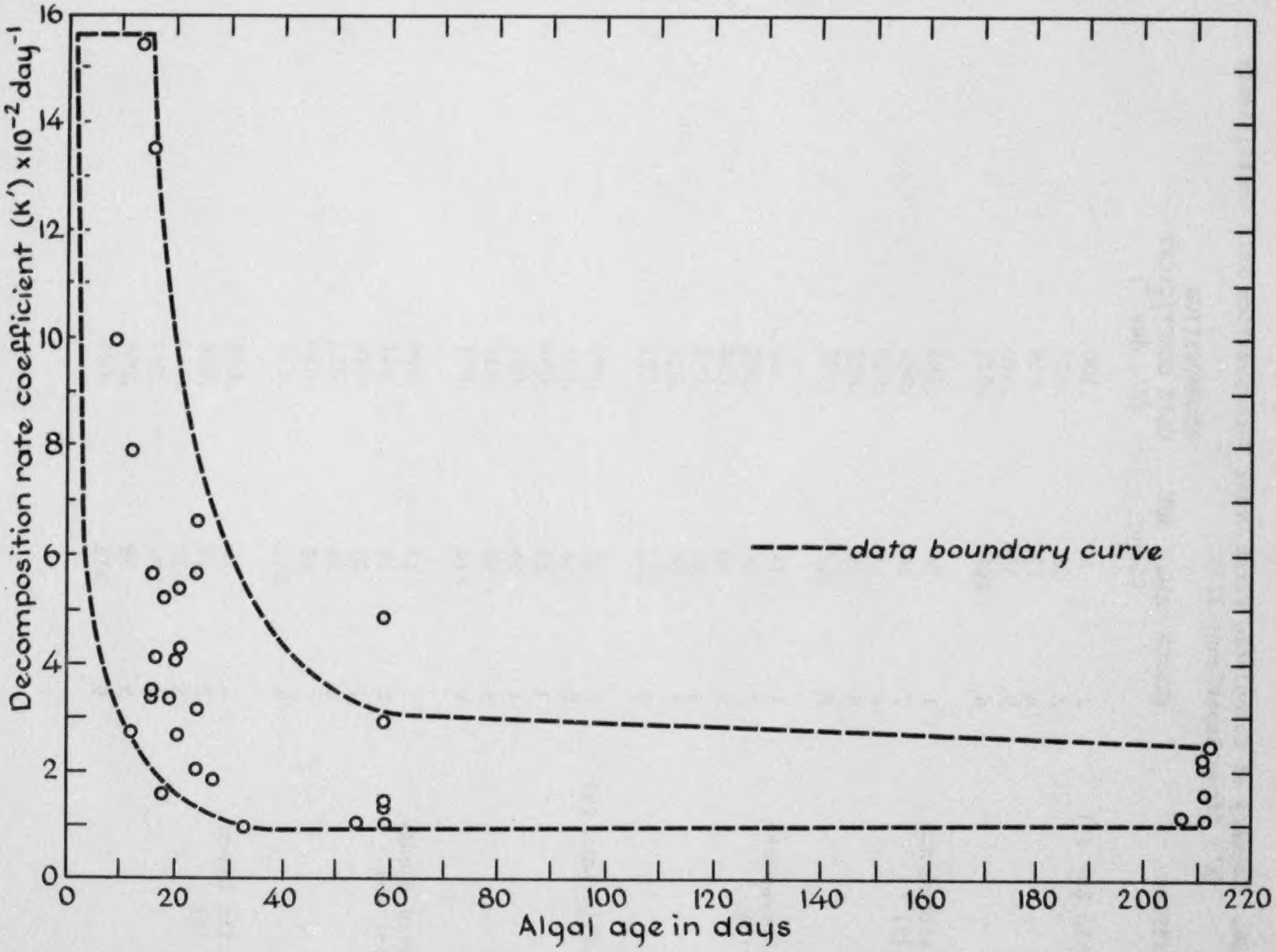

FIGURE 49. Effect of algal age on the first order rate of decomposition of simulated natural algal systems (Experiment III).

K' in Equation 9, predicted values for M were determined at each point in time for every measured value in the decomposition study of Experiment III. Table 37 summarizes the average error (per cent difference = $\frac{\text{Calculated Value - measured value}}{\text{Calculated value}}$ x 100) and standard deviation between the measured values and the predicted values using the postulated equation. The overall average per cent difference between the calculated and the measured values was found to be 12.6% with a variation of 5.9 per cent. This variability is nearly within the combined sampling and testing error of these studies. Note that the curves drawn in Figures 20 to 25 when the measured values are illustrated are the calculated curves using f given in Table 19 and K' given in Table 36. In most cases the data is described fairly well by first order decomposition kinetics even though the total organic mass varied over nearly a 50 fold range and was composed of widely varying algal communities grown in several different waters. The fact that most of these studies can be described by a first order relationship provides strong support for the use of Equation 9.

The observation that first order decay kinetics describes the process of naturally decaying algal systems has been made by several researchers (20,24). The observed rates of simulated natural algal decay, although derived from laboratory studies, also agree with the reported natural rates of decay. Most researchers have reported values for K_1 (Table 1) for natural algal decay as ranging from 0.04 to 0.08 per day. The rates observed in the simulated natural decomposition studies (Experiment III) varied from 0.0099 to 0.154 per day. Thus

Table 37. Error* in approximating the Decomposition Kinetics with the First Order Equation in Experiment III.

ALGAL SYSTEM	BRANCH	NUMBER MEASUREMENTS	AVERAGE % DIFFERENCE	STANDARD DEVIATION OF % DIFFERENCE
San Pablo Bay (1)	1	7	5.9	7.2
	2	6	13.2	17.8
	4	7	8.3	6.2
	5	6	8.3	16.7
	6	8	9.4	7.6
Tidal Sacramento River (2)	1	7	10.1	11.9
	2	5	8.6	10.4
	4	7	11.0	18.2
	5	5	8.2	2.8
	6	8	12.0	12.5
Fresh Sacramento River (3)	1	5	21.1	12.4
	2	7	11.2	26.3
	3	7	44.0	32.8
	4	7	9.4	9.6
	5	5	16.7	6.5
	6	8	10.4	8.2
Searsville Lake (4)	1	7	15.5	6.3
	2	8	14.2	10.8
	3	7	12.7	10.3
	4	6	27.5	22.8
	5	6	1.6	1.1
	6	9	4.8	2.6
Activated Sludge Effluent (5)	1	7	15.9	4.9
	2	8	17.7	5.4
	3	7	13.6	5.9
	4	7	8.5	8.6
	5	6	3.7	1.8
	6	9	4.1	3.4
Synthetic River Water (6)	1	7	20.4	13.7
	2	5	15.4	12.7
	3	7	10.6	13.1
	4	6	18.0	16.1
	5	6	19.0	12.8
	6	8	4.4	4.6

Overall average difference = 12.9%
Overall standard deviation of % differences = 5.9%

*Per Cent Difference = $\frac{\text{Calculated M} - \text{Measured M}}{\text{Calculated M}} \times 100$

although this study defines the rate of decomposition of algae over a wider range than previously reported, the values do fit in well with the previously observed values.

Figure 49 illustrates the effect of age on the decay rates found and shows that the rate of decay or the biodegradability is defined fairly well in a plot of the decay rate coefficients (of the biodegradable fraction) for all 35 different algal systems versus age of the system (days in diurnal light). This graph shows that the correlation of the rate of degradation to the age of the algal community is surprisingly good considering that these data are representative of many types of algal communities and water composition. It may be seen in Figure 49 and Table 36 that most algae studied by other researchers were relatively young with the oldest probably being not more than 20 to 30 days old.

With favorable seeding and temperatures it was shown that decay rates equal to and greater, in several cases, to those normally observed with rapidly degradable organic material such as domestic sewage could be achieved with algal systems. Table 29 of the Results Section summarizes some of the decay rates from the decomposition of seeded pure cultures and shows that decay rates two times higher than the maximum algal decay rates reported in the literature were not uncommon. Note that the rates in this table were determined by dividing the total average suspended solids present into the oxygen utilized over a certain time period. Thus if only the biodegradable COD were used to determine these decay rates the values could be more than twice that reported by other researchers. Minimum decay rates observed under conditions of pure culture, old age, or low temperature were usually around 0.01 per day.

The practical value of the hypothesis and the empirical data resulting from this study can be realized only if the data can be shown to be applicable to the general case of aerobic algal decomposition. In using Equation 9 the biodegradable fraction of algae and the rate of decomposition must be known. The effect of age on the decay rate has already been discussed. The refractory fraction of the simulated natural algal samples was also shown to be correlated closely with the age of the algal community in Table 19 and Figure 26 of the results. Young cells (0 to 20 days) appeared to be extremely resistant to biological breakdown and may be assumed to contain 50% or more refractory organic matter (maximum range 18.7 to 87.5); medium age cells (20 to 80 days old) may have a refractory organic fraction as low as 30% (maximum range 18.7 to 60); whereas very old cells (80 days or more) may have a stable fraction of 60% or higher (maximum range 42 to 74). Thus by knowing the algal age and then by using Figures 26 and 49 to determine f and K', Equation 9 can be used to calculate the average decomposition kinetics for algae in many types of waters.

If algal growth kinetics were similar to other microorganisms such as bacteria, one would expect algae to grow and decay rather quickly with on-set of a new species or genera of algae to utilize the decomposition products. With a rapid cycle such as this the refractory fraction of the algal community would increase with each new algal population. As long as growth continued the refractory material would continue to increase until the algal community had all the usable nutrients tied up in refractory organic material. As shown in Figure 28 in the Results Section, this fraction did build up with time in the studies of simulated natural conditions. After 211 days of growth the refractory organic material increased to 70% of the total organic material. This increase in refractory organic material was correlated with algal growth, but whereas a bacterial life cycle may be in the order of minutes (213) the algal cycle was apparently in the order of months, thus causing a slow accumulation of refractory organic material in the system. These growth kinetics emphasize the difference between micro-algae and bacteria and thus indicate that information should not be extrapolated from one area to the other.

Aging of an algal community is inseparable from nutrient depletion and in this sense is one of the controlling variables in algal decomposition. As the natural algal community ages the various genera of algae present in the community become nutrient deficient and deplete their storage products. This process causes the algae to become a less suitable substrate for the decomposers. The endogenous algal respiration may also fall to an insignificant level. During the aging of algal communities the morphology of the algal cells changed drastically. All algal cells went through a process of plasmolysis over a long period of time, causing the cells to shrink and become unidentifiable. This life form is more compact and protected and thus more resistant to decomposition.

The lipid composition of algal cells may increase to such high concentrations as an algal culture ages that it may be expected that this material would influence decomposition and nutrient regeneration. A review of nitrogen metabolism in algae indicated that the majority of algal species accumulate lipids when exposed to a nitrogen deficient environment (217). Table 22 in the Results Section shows the lipid content of old algal systems (branch 6) of six natural waters. These results indicate that where added nitrogen was provided (by nitrogen fixation in samples 4, 5, and 6) the lipid content remained at levels comparable to most biological material, i.e., less than 10%. But in the samples where little nitrogen fixation was observed, the lipid content of the algal systems increased to over 54% of the total dry weight.

There was little contrast between the cases of algal decay with a normal level and those with high concentrations of lipids. The rates of decay in all cases were very low with no significant difference in the amount or the character of the refractory organic material. However, in the three cases where a high lipid accumulation took place during growth, the lipids were degraded during decomposition leaving only about 10 per cent of the refractory material as lipid. Thus it did appear that most of the accumulated lipids were susceptible to biological decomposition.

It might seem that young algal systems should be more degradable than older systems and that the refractory fraction would increase as the algal community aged. The results from the chlorophyll study (Experiment VI) indicate a possible explanation for the resistance of young cells to biological decomposition. Alexander and co-workers (214,215) have clearly demonstrated that a controlling factor in the decomposition of certain fungi is the presence of a pigment called melanin. Thus it would not be unreasonable to expect that the pigments might affect the refractory organic fraction of young algae. From the data in Figures 43 to 46 it appears that the young cells with a relatively high chlorophyll concentration, although having a relatively high initial endogenous respiration rate, were also highly resistant to decomposition. The minimum in biodegradability occurred in all cases at a chlorophyll concentration between 0.2 and 0.4 per cent (dry weight).

The exact mechanism by which the chlorophyll might provide the algal cell with protection against the decomposing agents is not known. Chlorophyll is synthesized in laminar layers with a hydrophilic and a hydrophobic layer of lipoid type material between each layer (200). This layering might make the algal cell resistant to bacteria and animal attack.

A consideration of the formation of the refractory organic matter clearly indicates that most of it is associated with the algae and only a small quantity is contributed by the decomposers. Figure 28 shows that during the stationary period in natural water from samples #2 and #3 (Experiment III), the refractory material changed very little. However, in the simulated natural algal systems where additional fixed nitrogen caused growth to continue over the length of the study, the refractory fraction continued to increase. Since the rate of formation of new cells is slow,the formation of refractory organic matter is relatively slow. Thus the refractory fraction was found to increase from 30 to 40% up to 50 to 70% over a period of 211 days. When algal growth was limited, the algae remained in an apparent resting state in which there was little change in the refractory fraction over a period of up to 151 days. This also implies that as long as algae are exposed to the light, bacteria and animal growth did not consume a large amount of algae, since the refractory organic matter changed very little.

Further evidence indicating that the refractory organic material forms simultaneously with algal growth was obtained with the pure culture studies. Seeded young and old (difference of about 80 days) were stabilized and it was found that young cultures had refractory fractions similar to old cultures, but the quantity of refractory material was increased. Table 27 summarizes these results. Note that with Scenedesmus, the old cultures were more biodegradable than the young cultures, on a percentage basis, thus illustrating the paradoxical effect of aging in pure culture.

The results from the simulated natural studies (Experiment III) indicate that the interaction of algal species in nature produces a uniform and predictable fraction of refractory material in the algal system. In only one case was the algae found to be highly degradable. Here, acclimated bacteria and animals were added to pure cultures of Chlamydomonas and the refractory fraction was found to be only 8% after aerobic decomposition at 35°C for 5 months (at which time all activity had apparently ceased). This was, however, the only exception in over one hundred different studies of various types of algae under various conditions. There was also some evidence that some decomposition continued in algal samples maintained in the dark for periods of up to 700 days. These results indicate that biological decomposition of the "refractory fraction" may continue, but at extremely low rates, probably too low to be of any practical significance.

Effect of Temperature on Algal Decomposition

A change in temperature of a natural biological system may result in a large change in the overall rate of growth or decay of the system. The degradation of pure algae and seeded cultures of pure algae were studied at 4, 20, 25, and 35°C. The lower limit of temperature was chosen because of the predominance of this temperature in some natural water bodies during the winter. The results obtained at 4°C illustrated the effect of bacteria and animals on the decay mechanisms at this low temperature. Most of the results indicated that the regeneration of carbon in pure cultures of algae was extremely slow at 4°C with no nutrients appearing in solution. Seeded cultures at 4°C often had a negative carbon regeneration. That is, there was an increase in COD in the dark. This indicated that heterotrophic bacterial and algal (225,226) cell synthesis was going on at a rate higher than the respiration rate of the organic mass. Autotrophic mechanisms such as the oxidation of ammonia could increase the COD in the dark. Fixation of CO_2 by bacteria (219), the transformation of organic material resistant to the COD test to material that could be measured by the COD test, or experimental error could also account for the observed increase of the COD. Some combination of the above factors probably was operative.

It may be concluded that the oxygen demand from the decomposition process was close to zero at 4°C. The results from the BOD_5 tests at 4°C indicated the oxygen utilization was very low at this temperature (see Table 28). The nitrogen and phosphorus regeneration was also close to zero. However, caution should be observed in concluding that the biological activity is almost always completely inhibited at this temperature in nature. Some natural biological reactions may be relatively insensitive to temperature changes. That is, as the temperature decreases, the biological mass increases, with little overall change in the rate of oxygen consumption. In one instance at 4°C the forces controlling the decomposition of the diatom, Nitzschia closterium, were able to adapt to the low temperature and degraded the organic mass nearly as rapidly as at 20°C.

One major difference between natural decomposition and that observed in these experiments is that biological organisms are able to gradually adapt to major changes in temperature. In these studies all biological material was grown at 20°C and then subjected to the other temperatures. This sudden change of temperature yielded results which may be unlike that occurring in nature and so should be used with caution.

Studies at higher temperatures indicated a highly increased rate of decomposition in most cases. The temperature where maximum rates were observed varied between 25 and 35°C, thus indicating the variability of this type of study. Some algal systems apparently had optimum temperatures higher than 35°C whereas others had an optimum between 25 and 35°C. The variability of the results indicate the difficulties in generalizing about temperature effects with complex biological systems.

The maximum variation in the rate of decomposition with temperature in the range of 4 to 35°C expressed as Q_{10} values was from 0 to 3.0 as shown in Tables 28 and 30. Most studies however had Q_{10} values varying between 1.5 and 2.2. Although the effect of temperature on decomposition is variable, a Q_{10} value of 2.0 is appropriate for an approximation in the majority of cases.

Temperature also seemed to have an effect on the apparent refractory fraction of algal cultures. Although the magnitude varied, the refractory fraction at 35°C was always less at 20°C (Figure 40). The increase in biodegradability at higher temperatures is probably a reflection of the increased activity of all the biological mass, including the refractory organic material. Also, at higher temperatures it is possible that a higher mass of bacteria and thus a higher concentration of hydrolytic enzymes were made available in proportion to the algal mass available for degradation. It is also possible, if the chlorophyll does in fact provide a protective device against decomposition, that a temperature as high as 35°C could chemically hydrolyze the chlorophyll molecule. This would make the cell more susceptible to decomposition and might decrease the refractory organic fraction. The fact that the refractory fraction is dependent upon the temperature also indicates that the theory of "microbial infallability" when aided by favorable physical factors may hold when measured over a long period of time (several years).

Effect of Light, Algal Cell Viability, Anoxic Conditions on the Decomposition of Algae

Age of an algal community has been shown to be a major parameter that offsets the extent and rate of aerobic algal decomposition. The effect of parameters such as the viability of the cell, short term exposure to light, anoxic conditions, and high phosphorus and nitrogen conditions have been dealt with only briefly but all affect algal system decomposition.

The exposure of algal systems to 30 minutes or less of light per day had little overall effect on the decomposition process. However, a slight inhibition noted during the early stages of decomposition indicated that longer exposures of light would have caused a more significant effect. The influence of this variable is left for future research orientated towards application to the natural environment.

Anoxic conditions have been shown to have a drastic effect on aerobic bacterial and animal decomposition. The results from a short study on the effect of an anoxic environment on algal decomposition indicated that there was little difference in the refractory fraction resulting under aerobic and anaerobic conditions. Because of the limited nature of these studies, little can be concluded about the relative rates of decomposition, the potential problems arising from the hydrolysis of algal material in an anaerobic environment, and the subsequent stabilization of these soluble organic products. However, it is felt that the proposed controlling factors for algal decomposition would be altered very little under anaerobic conditions. If this were the case both the rates of conversion of particulate algal material to soluble organic material and the hypothesis for nitrogen and phosphorus regeneration (with proper modification of the decomposer synthesis factor) should hold for algal material under anaerobic conditions. This would be an area of great potential for future research because of the frequent occurrence of anaerobic conditions in nature.

The final variable that was considered as having some control on the decomposition of algal systems was the viability of the algal cells. It was found that algal cells killed by sudden release of a high hydraulic pressure were decomposed much more rapidly than the live cells. These results agree with numerous other studies. The viability and the integrity of the cell can therefore be assumed to be a major consideration in the rate of algal decomposition. However, cell integrity and viability had little effect on the refractory fraction of the algal system.

The above indicates algal decomposition is controlled by many parameters. The major portion of this research was spent in defining the reactions occurring in the dark aerobic environment under simulated natural conditions so that the results would be most applicable to engineering problems.

Mechanisms of Decomposition

The question as to the source of oxygen utilization during aerobic decomposition of algae is most important to answer since it is the key to understanding the decomposition process and nutrient regeneration. If the algal cell utilizes a large fraction of the total oxygen consumed it can be concluded that the algae themselves are responsible for most of the removal of the organic matter from the water. If either the bacteria or the animals consume an extremely high fraction of the total oxygen used in the decomposition process then most of the decomposition must be attributed to that group.

In general, it was shown in the literature review and in the experimental results of this study that endogenous algal respiration processes usually account for relatively little regeneration of carbon (as measured by COD), nitrogen and phosphorus. Table 30 summarizes the per cent of the total COD, N, and P regenerated over a period of several months in the pure culture studies. This period is equal to the time required for complete stabilization with bacteria and animals in other samples. Only in the cases of Chlamydomonas did the extent of regeneration of COD, N, and P consistently exceed 40%.

The regeneration of carbon by excretion of soluble organic matter is another process by which the algal cell can control both the rate and extent of decomposition. Both the literature review and the results from this study indicate that with the majority of species of algae the per cent of the organic matter excreted is small. The amount of measured extracellular carbon (except with Chlamydomonas) by pure algal cultures in the light and in the dark seldom exceeded 10% of the total carbon in the aerobic systems. Even in cultures where the dissolved organic carbon reached significant levels the dissolved nitrogen and phosphorus concentrations usually remained close to zero.

The amount of extracellular carbon measured in the light and dark heterogeneous cultures of the simulated natural studies (Experiments III, IV) was nearly always between 0 and 15 mg/l carbon, with a slight correlation between the total mass and the amount of soluble organic matter (see Figure 19). At high carbon concentrations of 300 to 400 mg/l, the soluble organic carbon increased to 20 to 30 mg/l in a few instances in the light cultures, but was less than 10% in most cases. After complete stabilization there was always a measurable soluble organic carbon content of between 3 and 10 mg/l C in all completely decomposed samples, indicating that a small portion of the excreted organic matter was resistant to decomposition. This agrees well with natural studies in unpolluted lakes and oceans where the soluble organic carbon is reported to be in the order of 2 to 4 mg/l carbon (218,219, 220,221,221).

The lack of evidence of a significant increase of soluble organic matter either during growth or decay of the simulated algal cultures does not indicate that excretion was absent. With the great variety of algal genera studied, some almost certainly must have excreted a large amount of soluble organic matter into solution. However, the fact that the concentration was always low when algae grew in the presence of the decomposers supports the hypothesis that the decomposer growth rate is nearly always controlled by the available substrate. This same argument applies to the appearance of soluble nitrogen and phosphorus in Zones B and C of Figure 5. In these two zones most of the soluble organic material given off by the algal material is used by the decomposers.

Regeneration of nitrogen and phosphorus will occur in Zone C but, in general, will not be correlated to a release of soluble organic carbon from algal material.

The conclusion that auto regeneration of C, N, and P by the algal cell is insignificant does not agree with some of the field studies completed with algae in dilute natural concentrations such as in the open ocean (92,238,207). It is quite possible that the difference between the results of this study and others lies in the fact that different species of phytoplankton may have been involved. Forms such as the flagellated Chlamydomonas might account for these differences. The changes studied in the ocean are usually measured over a time period much shorter than most of the analysis in this study. Thus rapid changes in this study might not be measured.

The concentrations of algae studied in this research ranged from a low of approximately 20 mg/l to a high of 1500 mg/l (dry weight suspended solids). Natural concentrations of algae in the ocean and in most pure waters are often lower than the minimum concentration studied here. Thus although it is reasonable to assume that the same mechanisms control algal decomposition in very dilute concentrations as at high concentrations, this has not been proven.

The same algae studied in pure culture were seeded with bacteria, and others were seeded with bacteria and animals in an attempt to clarify the question of the usage of oxygen by the various decomposing forces. Addition of bacteria to some pure cultures, and bacteria and animals to others indicated a slight difference between the decomposition process in pure cultures and in seeded cultures. However, the difference between bacterial, and bacterial and animal decomposition was negligible in all cases. Thus it was assumed that animals have little significant effect by themselves.

The decomposition of Chlorella in particular in pure culture and in cultures seeded with bacteria and animals shown in Figure 38 in the results illustrates the strong influence of the seed on algal decomposition. This supports the hypothesis that bacteria and microscopic animals have a major influence on algal decomposition.

Correlation of Chlorophyll to Algal Mass

Numerous attempts have been made to find a common denominator to relate the presence and activity of algae to some easily measurable variable. The logical choice has been the common chlorophyll pigments which most algae contain. There probably is a useful relationship between the oxygen production by phytoplankton in any natural water and the chlorophyll concentration because of the intimate relationship of chlorophyll with the biochemical pathways that are involved in the production of oxygen. However, the relation between chlorophyll concentration and other parameters is of questionable significance. Other researchers have used chlorophyll as a measure of the algal mass (228 229,230,231) and have even extrapolated it to be a measure of the oxygen usage by algae (231). For these reasons an attempt was made to determine the validity of such correlation. Figures 43 to 46 summarize the results of these studies and indicate a distinct lack of correlation between chlorophyll and algal mass. The chlorophyll concentration increased to a maximum (approximately 0.9 per cent of the dry weight) very rapidly in the early stages of growth and then declined almost as rapidly while the algae still appeared to be in a rapidly growing phase. This lack of correlation is supported by other researchers (231). A comparison between the rate of oxygen consumption (as determined by the standard BOD_5) and age (Figures 43 to 46) shows that there is also no correlation between the chlorophyll concentration and the rate of oxygen uptake of an algal system. Thus prediction from chlorophyll concentration should be viewed with caution.

REGENERATION OF NITROGEN AND PHOSPHORUS

The information presently available on the regeneration of nitrogen and phosphorus from the decomposition of algae and its associated organic material is based on empirical measurements. Researchers have reported the nitrogen remaining in the refractory organic material of phytoplankton (N_r) to vary between 16 and 50 per cent of that initially held in the particulate organic mass (19,20,99). The amount of phosphorus remaining in the refractory algal organic mass (P_r) is often reported as 33 per cent (93,99). The results from this study showed that regeneration of nitrogen and phosphorus from simulated natural algal communities and seeded pure algal cultures, varies from nearly 0 to 100 per cent of that initially held in the particulate organic material. Thus an hypothesis was desirable to define the parameters which control both the rate and the quantity of nitrogen and phosphorus regeneration.

It was assumed that the nutrients held in the refractory organic material (N_r,P_r) are composed of two fractions. One is the refractory algal material (N_{ra},P_{ra}) and the other is in the bacterial and microscopic animal residue (N_{rd},P_{rd}). The sum of these two fractions gives the total nitrogen and phosphorus which is unavailable for regeneration (N_r and P_r). Figures 50 and 51 illustrate calculated N_r and P_r) versus the observed values in the six major simulated natural algal systems. In all cases except for algae growing in sewage treatment plant effluent, the calculated values were on the average within 20% of the measured values. Note that the four points falling to the lower right of the line of perfect correlation were for young cultures of algae with high nitrogen and phosphorus concentrations. The predicted value was approximately half the measured value. Some of this variation in this region can be attributed to the fact that the nitrogen concentration was approaching the minimum accurately detectable level. However, the fact that less N and P were regenerated in these young cells than was predicted indicates that the assumption that young algae cells will have N_{ra} and P_{ra} concentrations of 3 and 0.3% (VSS) may be low for some algal cells at some stage during growth.

The difference between the predicted and measured value in the seeded pure culture studies (Experiment V) showed more variation than the values from the simulated natural algal studies (Figures 52 and 53). This is to be expected since in a pure culture the activity of the nitrogen and phosphorus is not affected by outside factors during growth as in the simulated natural environment. Another reason is because of the temperature effect on the refractory organic material (see Figure 40). All the values shown in Figures 50 and 51 were deter-

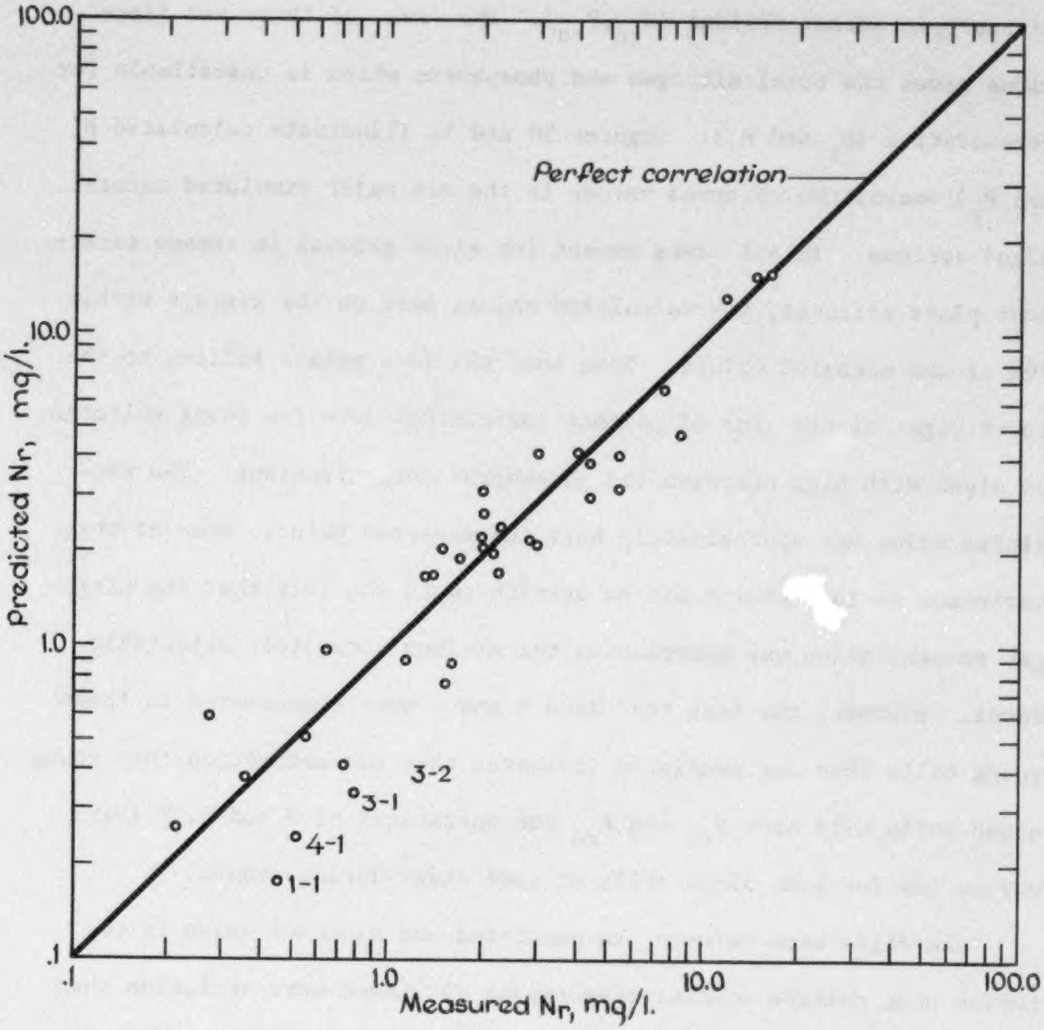

FIGURE 50. Comparison of predicted and measured values of nitrogen bound in the refractory organic material of simulated natural systems (Experiment III).

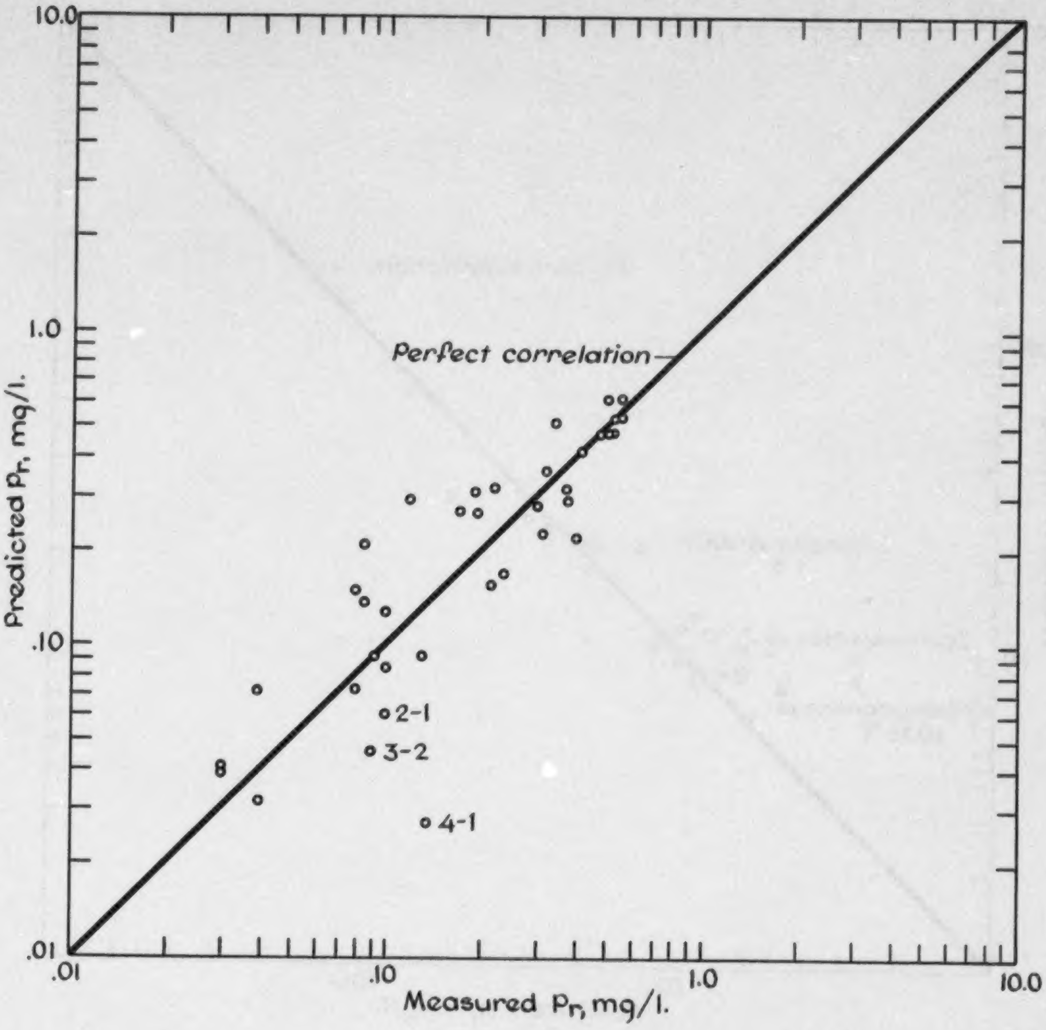

FIGURE 51. Comparison of predicted and measured values of phosphorus bound in the refractory organic material of simulated natural systems (Experiment III).

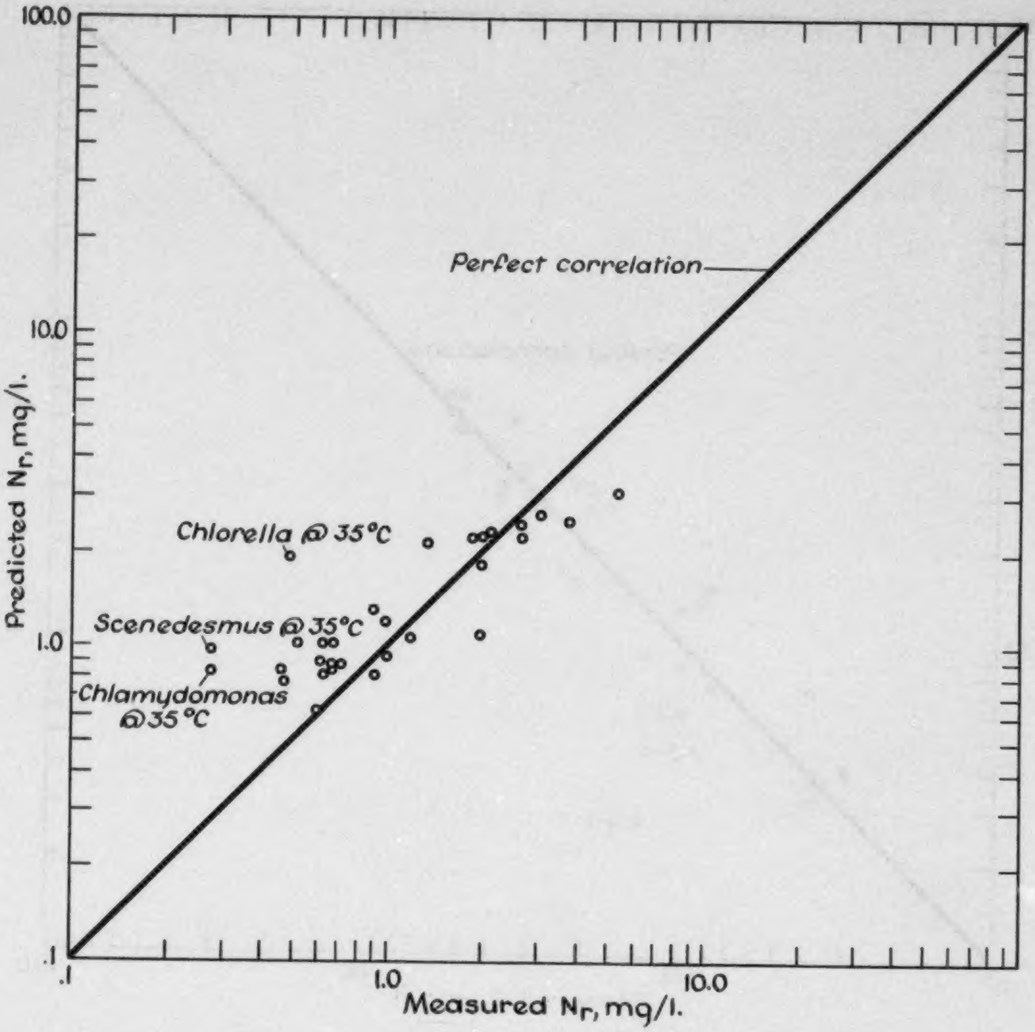

FIGURE 52. Comparison of predicted and measured values of nitrogen bound in the refractory organic material of seeded pure algal cultures (Experiment V).

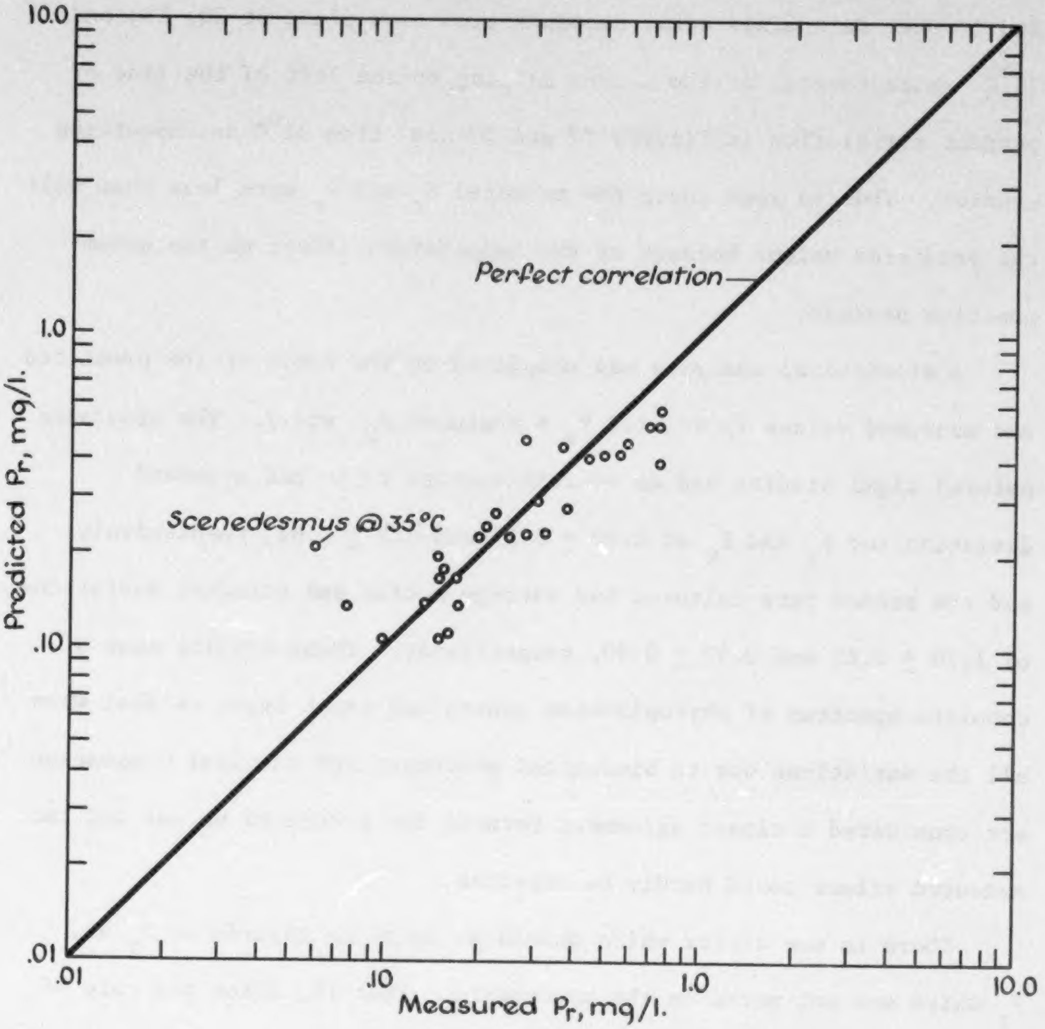

FIGURE 53. Comparison of predicted and measured values of phosphorus bound in the refractory organic material of seeded pure algal cultures (Experiment V).

mined after complete decomposition at 20°C whereas those in Figures 52 and 53 were determined after decomposition took place at 20, 25, and 35°C. Note several of the values falling to the left of the line of perfect correlation in Figures 52 and 53 are from 35°C decomposition studies. Thus in some cases the measured N_r and P_r were less than half the predicted values because of the temperature effect on the decomposition process.

A statistical analysis was completed on the ratio of the predicted and measured values (predicted $P_r \div$ measured P_r, etc.). The simulated natural algal studies had an overall average ratio and standard deviation for N_r and P_r of 0.90 ± 0.29 and 1.3 ± 0.62, respectively; and the seeded pure cultures had average ratios and standard deviations of 1.20 ± 0.61 and 0.97 ± 0.60, respectively. These results span a complete spectrum of phytoplankton genera and water types so that when all the variations due to biological processes and chemical phenomenon are considered a closer agreement between the predicted values and the measured values could hardly be expected.

There is one factor which should be noted in regards to N_r and P_r which was not noted in the hypothesis. That is, after the rate of biological decomposition of the organic material reached what appeared to be insignificant levels further regeneration of nitrogen and phosphorus occurred in some cases. It is not known whether this was a biologically induced release or whether it was caused by chemical hydrolysis of some of the nitrogen or phosphorus containing refractory organic material. In any case, it is of little significance in the practical sense.

Just as important as knowing the quantity of nutrients that may be returned to solution is the rate at which regeneration takes place. Although little can be said about zones A and B in Figure 5 it is postulated that in zone C regeneration follows the kinetics of carbon regeneration or decomposition. That is, the kinetics of nitrogen and phosphorus regeneration in this zone may be defined as:

$$\frac{d\,(N \text{ or } P)}{dt} = \alpha \frac{dM}{dt} \qquad \text{Eq (22)}$$

where α is a proportionality constant, N and P the particulate nitrogen and phosphorus concentrations, and M is the organic mass.

Note that in cases where luxurious nitrogen and phosphorus or other factors cause autolytic release of nutrients in zone A, this equation does not apply. Also this equation does not indicate the factors that control the length of zone B. Although the data in these experiments have indicated the controlling factors in zone A and B, analyses were not made frequently enough to clearly prove existence of these factors. Thus further definition of the rapid reactions occurring in zone A and the factors controlling the length of zone B in Figure 5 remain fertile areas for future research.

To further illustrate the kinetics of nutrient regeneration consider algal decomposition in sample 5 branch 1, Experiment III in which only zone C type regeneration existed. The decomposition kinetics in this case may be described as follows:

$$-\frac{dM}{dt} = K'(M - fM_o) \qquad \text{Eq (23)}$$

Then according to Equation 22 the kinetics of nitrogen and phosphorus regeneration should also be first order according to the following equations:

$$-\frac{dN}{dt} = \alpha(N - N_r) \qquad \text{Eq (24)}$$

$$-\frac{dP}{dt} = \alpha'(P - P_r) \qquad \text{Eq (25)}$$

Where N and P represent the total particulate organic nitrogen and phosphorus at any time, α and α represent the regeneration rate coefficients, and N_r and P_r are the nitrogen and phosphorus not available for regeneration. The solutions to the above two equations are as follows:

$$N = (N_o - N_r)\, e^{-\ t} + N_r \qquad \text{Eq (26)}$$

$$P = (P_o - P_r)\, e^{-\ 't} + P_r \qquad \text{Eq (27)}$$

Here, N_o and P_o equal the initial particulate organic nitrogen and phosphorus (mg/l) in the algal mass. The regeneration rate coefficients, α and α', were determined by graphical analysis of the data, , as in the determination of K', and were found to be 0.022 and 0.021 day^{-1} respectively. The calculated curves for nitrogen and phosphorus regeneration in sample 5-1 are shown with the measured values in Figure 54. Thus Equations 26 and 27 which were derived from the decomposition equations accurately describe nitrogen and phosphorus regeneration in

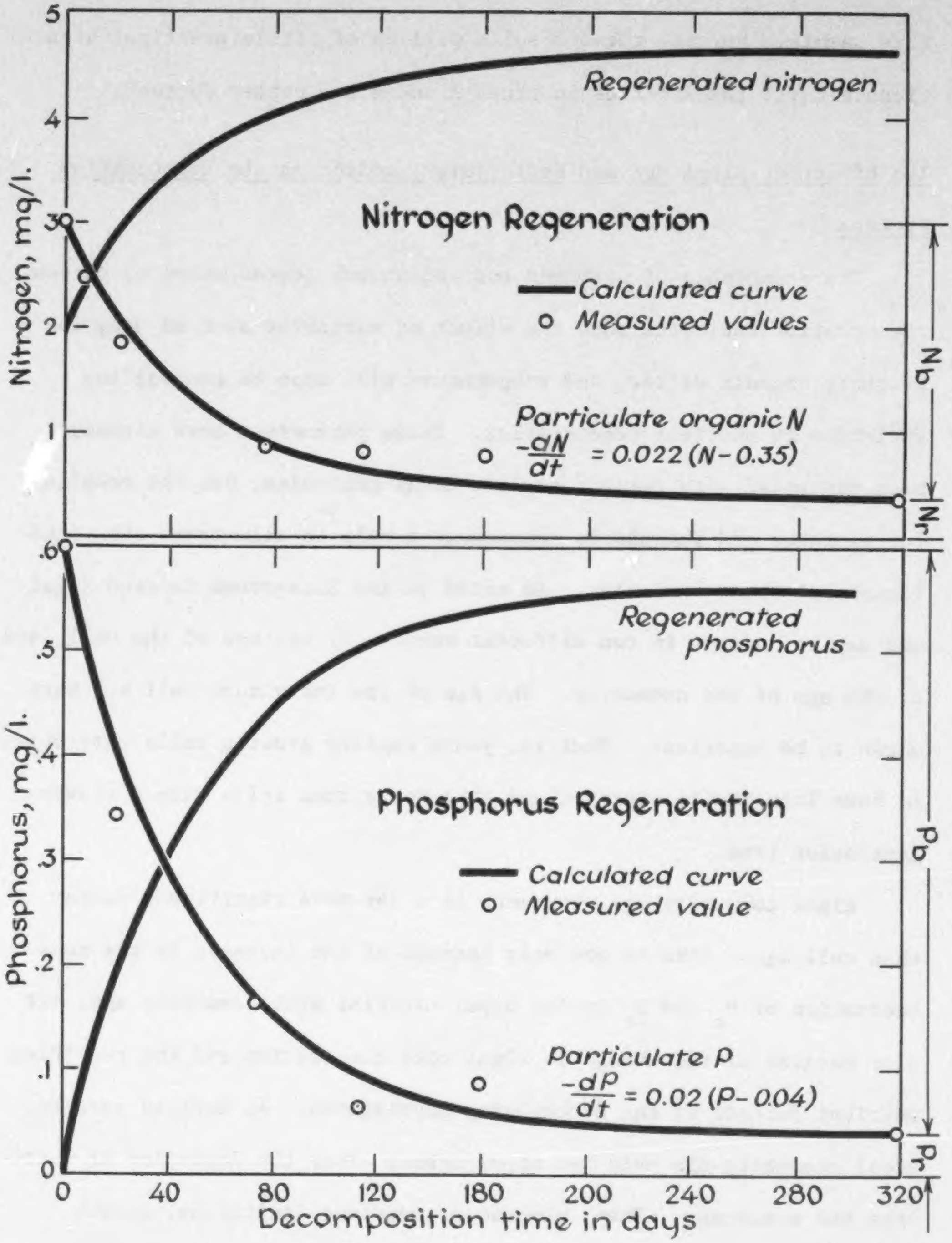

FIGURE 54. Comparison of calculated verses measured nitrogen and phosphorus regeneration.

this sample. However these results will be of little practical significance until the kinetics in zones A and B are better defined.

The Effect of Algal Age and Refractory Fraction on the Regeneration Process

The dependence of nitrogen and phosphorus regeneration on carbon regeneration indicates that the effect of variables such as age, refractory organic matter, and temperature will also be controlling variables in nutrient regeneration. These parameters have already been discussed with respect to the decay mechanism, but the results for nitrogen and phosphorus regeneration help to illustrate the significance of these variables. As noted in the Background Section algal age may be defined in two different ways: 1) the age of the cell, and 2) the age of the community. The age of the individual cell has been shown to be important. That is, young rapidly growing cells were shown to have less stable nitrogen and phosphorus than cells with a slower generation time.

Algal community age, however, is a far more significant factor than cell age. This is not only because of the increase in the concentration of N_r and P_r in the algal material with community age, but also because of the change in algal cell composition and the resulting nutrient demands of the decomposing populations. As defined earlier, algal community age only has significance after the depletion of nutrients has occurred. Then, because of nutrient limitation, growth gradually reaches a maximum. At this point the nutrients are usually distributed throughout the organic mass in a low concentration. The nitrogen fraction in the cells changes during the aging process from close to 10% (dry weight basis) in cells growing in a luxurious nutrient environment to just a little more than 1% at maximum yield; whereas the phosphorus may be as high as 3 to 4% initially and decreases to 0.1% or lower in older cultures.

However, even in the older cultures studied, where the nutrients were very low in concentration, between 30 and 40% of the nutrients were postulated to be released from the algal mass during decomposition, but were immediately absorbed and resynthesized by the decomposing populations so that no net regeneration occurred. This indicates that in older algal cultures with low concentrations of nitrogen and phosphorus, when 50 per cent of the algal material is decomposed, 50 per cent of the nutrients may be released from the algae only to be resynthesized into the refractory organic material of the decomposers. Thus the nutrients are not regenerated.

Since relatively little nutrients were available in relation to the biodegradable organic carbon with old algal cultures, serious questions could be raised as to whether the decomposer mass was limited by the availability of nitrogen or phosphorus. Information shown in Figure 27 and Table 33 indicated that added nutrients had little effect on the decomposition of older algal refractory organic material and also that added nutrients had little effect on the short term oxygen uptake (BOD_5) of algae with a wide variety of nutrient concentrations. Thus although the cultures in branch 6 of natural water samples (Figures 20 to 25) were almost certainly deficient in phosphorus, this did not appear to exert a controlling influence on the decomposition process.

Rather the controlling factor still appeared to be the rate of transition of algal material to a form available to the decomposers. The decomposer population may have been limited in some cases by the available nitrogen and phosphorus but the natural rate of decomposition of the algal material was so slow (even with excess nutrients) that a difference was not measurable. Because of the similarity in decomposition rates with a wide spectrum of algae ranging from those with luxurious nitrogen and phosphorus to those with a maximum deficiency, it may be concluded that there are nearly always enough nutrients available in the biodegradable algal material to satisfy the nutrient requirements of the decomposers.

Effect of Temperature on Nutrient Regeneration

The effect of temperature is very significant on nutrient regeneration only because of the increase in rate of carbon regeneration. In seeded cultures where rapid regeneration is possible the effect of temperature is extremely important. Figures 41 shows typical results of regeneration of nitrogen and phosphorus from Chlorella at 4, 20, 25, and 35°C. One might expect that the temperature extremes of 4 and 35°C might produce some regeneration of nitrogen and phosphorus through mechanisms other than those observed at more normal temperatures. Although the regeneration of nitrogen and phosphorus did not deviate from the characteristic of the carbon decomposition very often, different temperatures did cause significant differences in nutrient regeneration. It might be expected that algal autolysis at 4° or 35°C might be responsible for some regeneration and that carbon regeneration would not necessarily be carried out at 4°C, or that regeneration would occur at a slower rate than carbon regeneration at 35°C. At 4°C where little carbon regeneration was observed, little nitrogen and phosphorus regeneration took place. When at 4°C the decomposer mass acclimated to the low temperature and degraded the organic mass, nitrogen and phosphorus regeneration also took place. At 35°C when decomposition was extremely rapid, regeneration also reflected these high rates (see Figure 42 with Chlamydomonas). Thus nitrogen and phosphorus regeneration are dependent to a significant extent on carbon regeneration.

VII. ENGINEERING SIGNIFICANCE

The problems associated with the decomposition of algae are rapidly becoming some of the most pressing for the sanitary engineer. To date, however, few studies have been completed that allow a basic interpretation of the causes and effects of natural algal decomposition. This study has set forth hypotheses to explain carbon and nutrient regeneration along with supporting empirical data.

In general, the most significant outcome of this study is the finding that regardless of the species of algae or the chemical characteristic of the water (ranging from salt water to mountain spring water, from pristine pure water to highly polluted water) the growth and decay process is very similar under controlled conditions. This conclusion will allow one to apply the results from this study to most algal species or water conditions when modified by proper physical variable coefficients.

Summarized in the following section are some of the more significant results of this work and how they may apply to natural situations.

ALGAL YIELD

For over a hundred years people have been concerned with the effect of man on the aging of the water environment by increasing the nutrients available for plant growth. Numerous attempts to measure the ability of algae to grow under simulated natural conditions have not shown very promising results. The results of simulated natural algal growth in this study showed that studies to measure the algal yield of natural waters must be considered very carefully. It was shown that natural algal growth in the laboratory may be considered to be a self perpetuating phenomenon. That is, sufficient variety in the natural algal seed is probably always present to produce an algal bloom of one kind or another when sufficient nutrients are present. The more delicate and sensitive algae will bloom first, reach a stationary phase and then go into a declining phase. Before the amount of organic matter decreases significantly another type of algae will take over and grow to a maximum level and then start to decline. This cycle repeats itself over and over again until after a period of several years one may be able to determine the absolute maximum algal yield of any water. This yield is defined here as the total quantity of algae which can be produced on a given quantity of nutrients before all the available nitrogen and phosphorus is bound in the refractory organic material. However, this maximum algal yield is of limited practical importance.

The relationship of algal yield to the two major nutrients, nitrogen and phosphorus, is of much more significance. Many researchers have stated that either nitrogen or phosphorus or some ratio of these two nutrients limits algal growth. It was found that the minimum concentration of total organic nitrogen in the algae is in the order of 1.3% (dry weight) and has a variation from 0.84 to 2.6 with many different conditions and genera of algae, whereas the phosphorus concentration declined to values less than 0.06% (dry weight) before inhibition of growth was observed. The consistency of the minimum nitrogen concentration indicates that algal yield under favorable growth conditions is often limited by its nitrogen supply and that under such conditions the maximum algal yield (C, expressed as mg/l COD) may be predicted from the following expression:

$$C = aN \qquad \text{Eq (2)}$$

where a = 100 when the available nitrogen sources (N, expressed as mg/l nitrogen) do not include atmospheric nitrogen, and a = 50 when atmospheric nitrogen is available through nitrogen fixation. The expected error of prediction when a = 100 is around ± 20 per cent whereas the error when a = 50 is ± 40 per cent.

These above results indicate that, in order to limit algal growth in natural waters, control of the "available" nitrogen may be more important than control of the "available" phosphorus. This conclusion must be qualified since nitrogen may be an unlimited source in some cases where nitrogen fixation is taking place. However, in most flowing bodies of water and in many standing bodies the rate of nitrogen fixation is probably so low that the maximum algal yield is altered very little by the addition of nitrogen due to this process. In these instances studies to control man-made eutrophication should concentrate on the removal of nitrogen from waste water effluents. In other cases where it is thought that nitrogen fixation might contribute a significant quantity of nitrogen (in relation to the other "available" sources) studies to control eutrophication should consider the removal of P as well as the removal of nitrogen from waste water effluents.

ALGAL DECOMPOSITION

This study indicated that the effect of algal decay on water quality should not be measured on an algal cell basis but rather measurements should include all organic material that is synthesized as a result of the decomposition of algal cell material. Thus when an algal cell decomposes the total oxygen demand which results will include that from algal respiration, that from the respiration of the bacteria and animals that grew on the decomposition products of the algae, plus that used in eutrophic processes such as the oxidation of ammonia and nitrite-nitrogen.

Quantity of Algal Material Decomposed

Perhaps of most importance as far as the utilization of oxygen during decomposition is concerned is the refractory fraction of algal material, the extend of its formation, and its relationship to the kinetics of decomposition and nitrogen and phosphorus regeneration. In general the refractory fraction of algal material was found to vary in a predictable manner and equalled between 30 and 70 per cent of the COD as measured before decomposition in the dark began. This was true for most algal species at 20°C. That is to say that over a reasonable time period only between 30 and 70 per cent of the oxygen produced during photosynthesis will be required to decompose the algal organic matter which is synthesized. This statement should not be interpreted as indicating that algal growth must then always be beneficial to the oxygen budget of a water body. In most cases algae probably are decomposed in areas of the water body which are affected little by the addition of photosynthetic oxygen. In deep standing water bodies the algae formed in the eutrophic zone settle into lower stratified zones which are unaffected by the added photosynthetic oxygen. In flowing bodies of water, when the algae are young (with a low biodegradable fraction), the rate of oxygen production by photosynthesis may be many fold higher than the oxygen usage rate. But as the algae are transferred downstream the algal community ages and the rate of photosynthesis drops. With this increase in algal age there is also a significant increase in the quantity of biodegradable material available. Thus the photosynthetic oxygen is produced and possibly lost to the atmosphere or to other processes before it can be utilized in the stabilization of the algal material. Finally, in the bodies of water where there is a significant decrease in the velocity of flow (for example, in areas behind dams) the oxygen used during the decomposition process is, in general, without benefit from the oxygen which was produced during algal growth.

By considering the quantity of material which was resistant to decomposition and the decay rate coefficient, it was shown that the general second order equation for algal decomposition and the limiting first order case adequately described the kinetics of the two major types of natural algal decomposition. The observed rate of decay with the simulated natural algal studies could be used to predict "average" conditions whereas those rates observed with seeded pure cultures were felt to represent maximum rates of algal decomposition and thus may be used to predict the maximum effect that algal decomposition could have on a water body.

If the kinetics of algal decomposition can be described it is possible to calculate the effect of algae on the oxygen resources of any water body. For example, one method of improving the water quality of reservoirs has been shown to be mechanical circulation, the power requirements for which are high. The prediction of the oxygen usage by algal material will help to define the oxygen usage in an unpolluted standing water body such as a reservoir. With this factor know the power requirements to maintain aerobic conditions in a reservoir by mechanical mixing may be more accurately determined.

NITROGEN AND PHOSPHORUS REGENERATION

The release of nitrogen and phosphorus from algal cells to solution during algal decomposition may be called the translocation pollution potential of algae. That is, the nutrients that are regenerated in this manner are made available for more algal growth and the resynthesis of new algal material which can once more cause an oxygen demand on the water body. In order to define this problem both the quantity of nutrients which the algal material regenerates and the rate of regeneration must be known. The regeneration hypothesis set forth should help to predict both the rate and quantity of nitrogen and phosphorus regeneration, so that the translocation pollution potential of algae can be defined.

RELATIONSHIP OF CHLOROPHYLL TO ALGAL MASS AND OXYGEN USAGE

It was found that the chlorophyll content of algal cells varies significantly with age from a fraction of a per cent of the dry weight to over one per cent and then back to a fraction of the dry weight over a period of 10 to 20 days. This variation could not be directly correlated with any parameter of the algal organic matter except perhaps oxygen production which was not measured. Thus for any study in which chlorophyll is to be used as a correlating variable for algal decomposition a careful evaluation of the validity of this factor should be made.

ALGAL POND EFFLUENT EFFECT ON RECEIVING WATER BODY

Engineers have been concerned with the effect which algal pond effluents have on receiving water, but have not been able to give a satisfactory answer due to a lack of information on the oxygen usage and nutrient regeneration characteristics of algal material. The results of this study should help to predict both the maximum oxygen demand from the algae in the lagoon effluents and the rate and quantity of nitrogen and phosphorus which will be made available for further algal growth.

In some areas of the country a great concern for controlled eutrophication of water bodies might necessitate the use of expensive treatment processes to remove nitrogen and phosphorus from waste effluents. However, it should be possible to minimize or to completely eliminate the nutrient contribution of algae in algal pond effluents by growing algae so that their nutrient content would just be equivalent to the nitrogen and phosphorus required to meet the minimum needs of the decomposers. This could effectively eliminate all or much of the nitrogen and phosphorus regeneration potential in the algal pond effluent.

SETTING OF WATER QUALITY NUTRIENT STANDARDS

Throughout the United States there has been a dedicated drive to decrease pollution and increase the quality of all water bodies. One aspect of this country wide program has been the setting of water quality standards for most bodies of water. Some of these standards have included not only the minimum level for dissolved oxygen, but also minimum levels for nitrogen and phosphorus. In many instances the setting of these three standards has been governed by prior experience with the water body -- especially in regards to effects by algal growth. However, it is believed the results of this study will help in setting nitrogen and phosphorus standards for water bodies in terms of the oxygen resources available. The quantity of algal material which might arise from a given quantity of nutrients can be estimated and from this the potential oxygen usage may then be predicted. Note that in some cases oxygen usage may not be the controlling criteria since other factors such as aesthetics must enter into the setting of water quality standards.

A knowledge of the rate of nutrient regeneration will permit a determination of whether the rate of eutrophication is positive or negative in a lake or reservoir. That is, when the added nutrient sources are greater than the regenerated sources this causes a greater amount of algal growth and thus an increase in the rate of aging of the water body. As soon as the added nutrients become less than the regenerated nutrients the overall rate of aging of the water body will decrease. Depending on the physical characteristics it should be possible to predict the length of time that it would take bodies of water which have an "algal problem" (such as Clear Lake, California and Lake Erie) to revert to a desirable water body relatively free of man-made "algal problems."

VIII. CONCLUSIONS

Based on the results of this study, the following may be concluded:

ALGAL GROWTH:

1. The maximum quantity of organic matter produced by pure and heterogeneous algal systems under favorable diurnally lighted growth conditions show a strong dependency on the "available" nitrogen.

2. When the forms of nitrogen available for algal growth are limited to ammonia, nitrite, and nitrate the limiting ratio of COD/N for algal cultures will have a maximum range of 50 to 155 with a normal expected variation between 90 to 120.

3. When the forms of nitrogen available for algal growth include atmospheric nitrogen, the limiting COD/N value of algal cultures will be in the order of 50 with a maximum variation between 30 and 55.

4. Pure algal cultures growing under "favorable" conditions had observed maximum ratios of COD/P of 790, but heterogeneous algal cultures had COD/P ratios greater than 1350 in all cases, with one exceeding 2970.

5. Based on the limiting values of COD/N and maximum observed values of COD/P it may be stated that the nitrogen to phosphorus ratio in natural waters would have to be greater than 87 before the maximum quantity of algae produced would be limited solely by phosphorus.

AEROBIC DECOMPOSITION OF ALGAE:

1. In general, the rate of decay of algal derived organic material

is proportional to the concentration of biodegradable material present, and to the amount of biodegradable material which has been previously removed. The mathematical form for this expression is: $-dm/dt = K''mm_r$; where m represents the biodegradable material at any time, and m_r represents the biodegradable material removed at any time.

2. The most common type of natural algal decay occurs when the decomposers grow in proportion to and simultaneously with the algae. This is a special case of the above equation in which the first-order rate of decomposition of the biodegradable material is proportional to the concentration of the biodegradable material present.

3. The specific decay rate coefficient (K') for the first order decay equation varies between 0.0099 and 0.15 per day in a well defined manner with age of the algal system (8 to 211 days).

4. The biodegradable fraction of algae is nearly always less than 70 per cent (initial COD) with a maximum range of 13 to 88 per cent. The physiological characteristics of unicellular algal cells causes a high refractory fraction of 60 to 87 per cent in young algal cells (8 to 20 days old), with a subsequent decrease caused by nutrient depletion in the medium to around 40 per cent, and then an increase once more to over 50 per cent as the aging effect becomes more pronounced (54 to 211 day old algal cultures).

5. The decomposition of algae is nearly completely inhibited at $4^{o}C$, but increases in rate between 20^{o} and $35^{o}C$, with some inhibition noted at $35^{o}C$.

NITROGEN AND PHOSPHORUS REGENERATION:

1. The nitrogen and phosphorus held in the refractory organic matter of completely decomposed algal cultures is composed of two fractions: 1) the nitrogen and phosphorus held in the refractory algal material, and 2) the nitrogen and phosphorus held in the refractory bacterial and animal material.

2. The nitrogen and phosphorus held in the refractory algal material varies from a minimal concentration in the refractory material of young algal cells to a concentration similar to that in the older algal mass prior to decomposition. The dividing point between these may be taken as that level when the nitrogen is approximately equal to 3 per cent of the volatile algal solids.

3. Little nitrogen and phosphorus regeneration occurred in pure cultures in the dark (except with Chlamydomonas), but with seeded algal systems the quantity regenerated varied from close to 100 per cent to zero per cent depending on the age of the algal system and the initial concentration of the nitrogen and phosphorus in the algal particulate material.

IX. SUGGESTIONS FOR FUTURE WORK

Based on the findings of this study, the following topics are suggested as possible subjects for future work.

1. The incorporation of parameters such as natural lighting, settling, and other physical parameters into the algal yield, algal decomposition, and nutrient regeneration hypotheses.

2. The determination of the decomposition and nutrient regeneration kinetics on a natural diurnal basis.

3. The determination of what forms of nitrogen and phosphorus are "available" and under what conditions these forms become "available" for algal growth.

4. The further investigation of the dependence of the yield of all natural algal genus on the nitrogen and phosphorus concentrations.

5. The determination of the algal decomposition and nutrient regeneration kinetics under anaerobic conditions.

6. The further investigation of the mechanisms controlling the formation and resistance of the refractory algal material.

7. The further definition of nutrient regeneration. In particular to define: the rapid reactions occurring during the first several days of decomposition, and the factors which cause inhibition of nutrient regeneration during the early stages of decomposition in some cases.

8. The extension of the nutrient regeneration hypotheses to other chemical elements of sanitary engineering significance such as manganese and iron.

BIBLIOGRAPHY

1. Stewart, K.M. and Rohlich, G.A. Eutrophication - A Review. State Water Resources Control Board, California. Publication No. 34. 1967

2. Skulbert, O.M. "Algal Problems Related to the Eutrophication of European Water Supplies, and a Bio-Assay Method to Assess Fertilizing Influences of Pollution on Inland Waters." Algae and Man. Edited by D.F. Jackson. Plenum Press. 1964.

3. Oswald, W.J. and Goluke, C.G. "Eutrophication Trends in the United States - A Problem?" Water Pollution Control Federation Journal 38. (6) (June, 1966). 964-975.

4. Fruh, E.G. "The Overall Picture of Eutrophication." Water Pollution Control Federation J. 39. (9) (September, 1967). 1449-1463.

5. McCarty, P.L. et al. (Task Group 2610-P). Task Group Report: "Sources of Nitrogen and Phosphorus in Water Supplies." American Water Works Association. 59. (3) (March 1967). 344-366.

6. Cronin, I.E. and Flemer, D.A. "Energy Transfer and Pollution." In: Pollution and Marine Ecology. Edited by T.A. Olson and F.J. Burgess. (1967). 171-162.

7. Knopp, H. "Investigation of the Oxygen Potential of River Plankton." Schweiz. Z. Hydrol. 22. 152-166.

8. Winberg, G.G. and Sivko, T.N. "The significance of Photosynthetic Aeration on the Oxygen Balance of Polluted Waters." International Journal of Air and Water Pollution. 6. (1962). 267-275.

9. Bartsch. "Algae in Relation to Oxidation Processes in Natural Waters." The Ecology of Algae. Pymatuning Symposium in Ecology. Edited by C.A. Tyron, Jr. and R.T. Hartman. (June 18, 19, 1959).

10. O'Connell, R.L. and Thomas, N.A. "Effect of Benthic Algae on Stream Dissolved Oxygen." ASCE Journal San. Eng. Div. 91. No. SA.3. (June, 1965) 1-17. And Closure in 92 No. SA.5. (October, 1966). 95-99.

11. Wisniewski, T.F. "Algae and Their Effects on D.O. and B.O.D." Water and Sewage Works. 105. (June, July 1958). 235-241, 300-305.

12. Bartsch, A.F. "Algae and Their Effects on D.O. and B.O.D." Water and Sewage Works. 105, (August 1958). 339-341.

13. Dunbar, K.S. Personal Communication. Department Water Re-Sources, California. December, 1967.

14. Stumm, W. and Morgan, J.J. "Stream Pollution by Algal Nutrients." Trans. 12th Annual Conference on Sanitary Engineering. Univ. of Kansas Eng. Bulletin. 1962. No. 50.

15. Alexander, M. "Biodegradation: Problems of Molecular Recalcitrance and Microbial Fallibility. Advances in Applied Microbiology. 7. Edited by Wayne W. Unbreit. Academic Press, (1965). 35-80.

16. McKinney, R.E. "Benthic Algae on Stream Dissolved Oxygen." ASCEJ San. Eng. Div. 91, No. SA 5. Part 1, (October, 1965). 77-78.

17. Kuntz, R.R. and Forney, Jr., C. "Metabolic Energy Balances in a Total Oxidation Activated Sludge System." Sewage and Industrial Wastes. 31, (1959). 819-826.

18. Washington, D.R. and Symons, J.M. "Volatile Sludge Accumulation in Activated Sludge Systems." Water Pollution Control Federation Journal. 34, (1963). 283-291.

19. Kleerekoper, H. "The Mineralization of Plankton." J. Fish Res. Bd. Can. 10. (5), (1953). 283-291.

20. Skopintzen, B.A. and Broock, E.A., "A Study of the Oxidation Processes Occurring in Water During the Aerobic Decomposition of Phytoplanktons," Mikrobiology, Moscow, 9, (1940),(pp. 595-607). Article in Russian.

21. Golueke, C.G., Oswald, W.J., and Gotaas, H.B. "Anaerobic Digestion of Algae." Applied Microbiology. 5, (1957), 47-55.

22. Golueke, C.G. and Oswald, W.J. "Biological Conversion of Light Energy to the Chemical Energy." Applied Microbiology. 7, (1959), 219-227.

23. Cramer, M.L. and Myers, Jr. "Effect of Starvation on the Metabolism of Chlorella." Plant Physiol. 24. (1949), 255-264.

24. Fitzgerald, G.P. "The Effects of Algae BOD Measurements." Water Pollution Control Federation J. (December, 1964), 1524-1542.

25. Allen, M.B, Fitzgerald, G.P., and Rohlich, G.A. "The Effect of Dilution Media on the BOD of Algae." *Water Pollution Control Federation J*. *36*. (8), (August, 1964), 1049-1056.

26. Gibbs, M. "Respiration." In: *Physiology and Biochemistry of Algae*. Edited by R.A. Lewin. Academic Press. (1962), 61-86.

27. Kratz, W.A. and Myers, J. "Photosynthesis and Respiration of Three Blue-Green Algae." *Plant Physiol*. 30. (1955), 275-280.

28. Milojeric, M. "Contributions of Steam-Sterilized Algae to BOD." *Water Pollution Control Federation J*. (1967), 1203-1210.

29. Syrett, P.J. "The Assimilation of Ammonia by Nitrogen-Starved Cells of *Chlorella vulgaris*. Part 1. The Correlation of Assimilation with Respiration." *Annals of Botany N.S.* *17*, (1953), 1-19.

30. Fogg, G.E. *The Metabolism of Algae*. Methuen and Co., Ltd. London.(1953).

31. Nalewajko, C. "Photosynthesis and Excretion in Various Planktonic Algae." *Limnol*. *and Oceanography*. XI. (1), (January, 1966) 1-10.

32. Hellebust, J.A. "Excretion of Some Organic Compounds by Marine Phytoplankton." *Limno*. *and Oceanography.* *10*, (1965), 192-206.

33. Fogg, G.E., Nalewajko, C., and Watt, W.D. "Extracellular Products of Phytoplankton Photosynthesis." *Proc*. *Roy*. *Soc*. *Lond*. *B 162*, (1965), 517-534.

34. Allen, M.B. "Excretion of Organic Compounds by *Chlamydomonas*." *Archiv fur Kikrobiologie*. *24*. (1956), 163-168.

35. Round, F.E. *The Biology of the Algae*. Published by Edward Arnold Ltd. (1965).

36. Odum, E.P., and Odum, H.T. *Fundamentals of Ecology*. Published by W.B. Saunders Company. Philadelphia, (1963),

37. Kiss, A.E. "Microbiology and the Chief Problems in the Black Sea." *Deep Sea Research*. *5*. (1959) 193-200.

38. Birge, E.A. and Juday, C. "Particulate and Dissolved Organic Matter in Inland Lakes." *Ecological Monographs*. 4, (1934), 440-474.

39. Ward, C.H., Moyer, J.E., and Vela, G.R. "Studies on Bacteria Associated with *Chlorella Pyrenoidosa* TX71105 in Mass Culture." *Developments in Industrial Microbiology*. 6. Edited by American Institute of Biological Sciences, (1964).

40. Jorgensen, C.B. Biology of Suspension Feeding. Peagamon Press. (1966).

41. Teal, J.M. "Community Metabolism in a Temperate Cold Spring." Ecol. Monogr. 27. (1957), 283-302.

42. Odum, H.T. "Trophic Structure and Productivity of Silver Springs, Florida." Ecol. Monogr. 27. (1957), 55-112.

43. Collins, V.G. "Planktonic Bacteria." J. Gen. Mocrobiol. 16. (1957), 268-272.

44. Oppenheimer, C.H., and Vance, M.H. "Attachments of Bacteria to the Surface of Living and Dead Microorganisms in Marine Sediments (Attachment of Bacteria to Microorganisms)." Zeitschrift fur Allg. Mikrobiologie 1. (1) (1960), 47-52.

45. Waksman, S.A., Stokes, J.L., and Butler, M.R. "Relation of Bacteria to Diatoms in Sea Water." J. Mar. Biol. Assoc. 22. (1937), 359-373.

46. James, A.M. "The Electrochemistry of the Bacterial Surface." Progress in Biophysics. Edited by J. Butler and B. Katz. 8. (1957), 95-142.

47. Golueke, C.G. and Oswald, W.J. "Harvesting and Processing Sewage-Grown Planktonic Algae." Water Pollution Control Federation J. 37. (4), (April, 1966), 471-498.

48. Parker, C.D. "Microbiological Aspects of Lagoon Treatment." Water Pollution Control Federation J. 34, (1962), 149-161.

49. Mottoni, R.H.T., Myrick, H.N. and Keller, Jr., E.C. "Application of Industrial Photosynthesis Process to Waste-Water Renovation Systems." 20th Industrial Waste Conference. Purdue University. Eng. Bull. Series No. 118. (1966).

50. Spoehr, H.A., Smith, J.H.C., Strain, H.H., Milner, H.W., and Hardin, G.J. "Fatty Acid Anti-bacterials from Plants." Carnegie Inst. Wash. Pub. (1949).

51. Nielsen, E. Steeman. "The Production of Antibiotics by Plankton Algae and its Effect upon Bacterial Activities in the Sea." Deep Water Research, Suppl. to vol. 3. (1965), 281-286.

52. Davidson, F.F. "Antibacterial Activity of Extracts Obtained from Blue-Green Algae." Proceedings of the Seventeenth Industrial Waste Conference. Eng. Bull. Purdue University. (May 1, 2, 3, 1962).

53. Proctor, V.W. "Studies of Algal Antibiosis Using *Haematoccus* and *Chlamydomonas*." Limnol. and Oceanogr. 2. (1957), 125-139.

54. Pratt, R., *et al*. "Chlorellin, and Antibacterial Substance from *Chlorella*." *Science*. *99*, (1944), 351-352.

55. Sieburt, J.M. "Role of Algae in Controlling Bacterial Populations in Estuarine Waters." Comm. Inter. Pour L'exploration Scientifique de la mer Mediterranee. *Symposium de Monaco*. (1965), 217.

56. Pratt, R. "Studies on *Chlorella vulgaris*. VI. Retardation of Photosynthesis by a Growth Inhibiting Substance from *Chlorella vulgaris*." *Amer*. *J. Botany*. 30. (1943), 32-33.

57. Pratt, R. "Studies on *Chlorella vulgaris*. VII. Influence of the Age of the Culture on the Rates of Photosynthesis and Respiration." Amer. J. Botany. 30. (1943), 404-407.

58. Leone, D.E. "Growth of *Chlorella pyrenoidosa* in Recycled Medium." Appl. Microbiol. 11. (1963), 427-429.

59. Phillipson, J. *Ecological Energetics*. Edward Arnold Publishers, Ltd. (1967).

60. Fish, G.R. "Digestion in *Tilapia esculenta*." *Nature*. *167*. (1951), 900-901.

61. Cushing, D.H. "The Effect of Grazing in Reducing the Primary Production: A Review." *Intern*. *Council for the Study of the Sea*. *Rapports et Process-Verbaux*. 144. (1958), 149-154.

62. Nauwerck, A. "Die Beziehungen Zwischen Zooplankton und Phytoplunkton Im See Erkan." *Symbolae Botanical Upsalienses*. 17. (5), (1963), 1-163.

63. Lund, J.W.G. "Studies on *Asterionella formosa Hass*. II. Nutrient Depletion and the Spring Maximum." Part II. Discussion. *J*. *Ecol*. *38*. (1950), 15-35.

64. Pennak, R.W. "The Dynamics of Fresh-Water Plankton Populations." *Ecol*. *Monogr*. 16. (1946), 339-356.

65. Lund, J.W.G. "Studies on *Asterionella*. 1. The Origin and Nature of the Cells Producing Seasonal Maxima." *J*. *of Ecology*. *37*. (1949), 389-419).

66. Deevey, G.B. "The Zooplankton of Tisburg Great Pond." *Bingham Oceanogr*. *Collection*. *12*. (1), (1948), 1-44.

67. Riley, G.A., Stommel, H., and Bumpus, F.F. "Quantitative Ecology of the Plankton of the Western North Atlantic." *Bingham Oceanogr*. Collection 12. (3), 1949),1-169.

68. Sladecek, V. "A Note on the Plytoplankton - Zooplankton Relationship." Ecology. 39. (3), (July, 1958), 547-549.

69. Bainbridge, R. "Studies on the Interrelationships of Zooplankton and Phytoplankton. " *J*. *Mar*. *Biol*. *Assoc*. *U*.*K*. *32*. (1953) 385-447.

70. Lefevre, M. "L'Utilisation des Algues d'Eau Douce Par Les Claduceres." *Bulletin Biologique de la France et de las Belgique*. *76*. (1942), 250-276.

71. Provasoli, L., Shiraishi, K., and Lance, J. R. "Nutritional Idiosyncracies of *Artemia* and *Tigriopers* in Monoxenic Culture." *Ann*. *N*. *Y*. *Acad*. *Sci*. *77*. (1950), 250-261.

72. Slobodkin, L.B. *Growth and Regulation of Animal Populations*. Holt, Rinehart and Winston, Publishers. 1962.

73. Morris, R.W. "Some Aspects of the Problem of Rearing Marine Fishes." *Bulletin Inst*. *Oceanogr*. *Monaco*, *1082*. (1956), 1-61.

74. Cowell, B.C. "The Copepods and Cladocera of a Missouri River Reservoir: A Comparison of Sampling in the Reservoir and the Discharge." *Limnol*. *and Oceanog*. *12*. (1) (January 1967). 125-137.

75. Williams, L.G. "Dominant Planktonic Rotifers of Major Waterways of the United States." Limnol. and Oceanog. 11 (1) (January 1966).

76. Lackey, J.B. "Nutrient and Pollutant Response of Estuarine Biotas." *Proceedings of the National Symposium on Estuarine Pollution*. Stanford University. August 1967.

77. Marshall, S.M. and Orr, A.P. "On the Biology of *Calanus finmarchicus*. Food Uptake, Assimilation and Excretion in Adult and Stage V. *Calanus*." *J*. *Mar*. *Biol*. *Ass*. *U*. *K*. *34*. (1955) 495-529.

78. Marshall, S.M. and Orr, A.P. "Experimental Feeding of the Copepod *Galanus Finmarchicus* (Gunner) on Phytoplankton Cultures Labeled with Radioactive Carbon 14_C." *Deep Sea Research*. Supplement to Vol. 3. (1955), 110-114.

79. Gibor, A. "Some Ecological Relationships Between Phyto and Zooplankton." *Biological Bulletin*. *III*. (1956), 230-239.

80. Lasker, R. "Utilization of Organic Carbon by a Marine Crustacean: Analysis with Carbon-14." *Science*. *131*. (1960), 1098-1100.

81. Richman, S. "The Transformation of Energy by Daphnia pulex." *Ecol*. *Mon.* *28*. (1958) 273-291.

82. Comita, G.W. "Oxygen Uptake in Diaptomus Siciloides Lilljeborg." Notes and Comments In: *Limnol*. *and* *Oceanog*. *10*. (1965).

83. Corner, E.D.S., Cowey, C.B., and Marshall, S.M. "On the Nutrition and Metabolism of Zooplankton." *J*. *Mar*. *Biol*. *Ass*. *U.K*. *47*. (1967) 259-270.

84. Conover, R.J. "Factors Affecting the Assimilation of Organic Matter by Zooplankton and the Question of Superfluous Feeding." *Limnol*. *and* *Oceanog. 11*. (1966) 346-354.

85. Johannes, R.E. and Satomi, M. "Composition and Nutritive Value of Fecal Pellets of a Marine Crustacean." *Limnol*. *and* *Oceanog*. *11.* (1966) 191-197.

86. Conover, R.J. "Assimilation of Organic Matter by Zooplankton." *Limnol*. *and* *Oceanog*. *11*. (1966), 338-345.

87. L.B. Slobodkin. "Population Dynamics in *Daphnia* *obtusa* *Kurz*." *Ecol*. *Monogr*. *24*. (1954), 69-88.

88. Slobodkin, L.B. "Energy in Animal Ecology." *Advances* *in* *Ecological* *Research*. *1*. (1962), 69-102.

89. Fitzgerald, G.P. and Rohlich, G.A. "An Evaluation of Stabilization Pond Literature." *Sewage* *and* *Industrial* *Wastes*. *30*. 10. (October 1958), 1213-1224.

90. Mackenthem, K.M. "A Review of Algae, Lake Weeds, and Nutrients." *Water* *Pollution* *Control* *Federation* *J*. *34*. (1962), 1077-1085.

91. Hutchinson, C.E. *A* *Treatise* *on* *Limnology*. *Vol*. *II*. *Introduction* *to* *Lake* *Biology* *and* *the* *Limnoplankton*. Published by John Wiley and Sons, Inc. (1967)

92. Cooper, L.H.N. "The Rate of Liberation of Phosphate in Sea Water by the Breakdown of Plankton Organisms." *J*. *Mar*. *Biol*. *Assoc*. *U*. *K*. *20*. (1935) 197-200.

93. Von Brand, T., Rakestraw, N.W., and Renn, C.E. "The Experimental Decomposition and Regeneration of Nitrogenous Organic Matter in Sea Water." *Biological* *Bulletin*. 72. (1937), 165-175.

94. Von Brand, T., Rakestraw, N.W., and Renn, C.E. "Further Experiments on the Decomposition and Regeneration of Nitrogenous Organic Matter in Sea Water." *Biological Bulletin*. *77*. (1939), 285-296.

95. Von Brand, T., Rakestraw, N.W. "Decomposition and R egeneration of Nitrogenous Organic Matter in Sea Water. III. Influence of Temperature and Source and Condition of Water." *Biological Bulletin*. *79* (1940), 231-236.

96. Von Brand, T., and Rakestraw, N.W. "Decomposition and Regeneration of Nitrogenous Organic Matter in Sea Water. IV. Interrelationship of Various Stages: Influence of Concentration and Nature of Particulate Matter." *Biological Bulletin*. *81*. (1941) 63-69.

97. Von Brand, T., and Rakestraw, N.W. and Zabor, J.M. "Decomposition and Respiration of Nitrogenous Organic Matter in Sea Water. V. Factors Influencing the Length of the Cycle: Observations upon the Gaseous and Dissolved Organic N." *Biological Bulletin*. *83*. (1942), 273-282.

98. Goldeberg, E.D., Walker, T.J., and Whisenand. "Phosphate Utilization by Diatoms. *Biological Bulletin*. *101*. (1951), 274-284.

99. Grill, E.V. and Richards, F.A. "Nutrient Regeneration from Phytoplankton Decomposition in Sea Water." *Journal of Marine Research*. *22.1* (1964), 51-69.

100. Ketchum, B.H. "The Development and Restoration of Deficiencies in the Phosphorus and Nitrogen Composition of Unicellular Plants." *J. of Cellular and Comparative Physiology*. 13. (1939), 373-381.

101. Ketchum, B.H. "The Absorption of Phosphate and Nitrate by Illuminated Cultures of *Nitzschia Closterium*." *Am. J. of Botany*. *26*. (1939), 399-407.

102. Syrett, P.J. and Fowden, L. "The Assimilation of Ammonia by Nitrogen-Starved Cells of Chlorella vulgaris. III. The Effect of the Addition of Glucose on the Products of Assimilation." *Physiol. Plant*. *5*. (1952) 558-566.

103. Harvey, H.W. "Synthesis of Organic Nitrogen and Chlorophyll by *Nitzschia closterium*." *J. Mar. Biol. Assoc. U. K.* *31*. (1953), 477-487.

104. Harvey, H.W. "Note on the Absorption of Organic Phosphorus Compounds by *Nitzschia closterium* in the Dark." *J. Mar. Biol. Assoc. U. K.* *31*. (1953), 475-476.

105. Redfield, A.C. "On the Proportions of Organic Derivatives in Sea Water and Their Relation to the Composition of Plankton." James Johnson Memorial Volume, University Liverpool Press. (1934), 176-192.

106. Riley, G.A. "Oxygen, Phosphate and Nitrate in the Atlantic Ocean." *Bull. Bingham Oceanog. Coll.* *13*. (1), (1951), 1-126.

107. Cooper, L.H.N. "On the Ratio of Nitrogen to Phosphorus in the Sea." *J. Mar. Biol. Assoc. U.K.* *22*. (1938), 177-182.

108. Barlow, J.P. and Bishop, J.W. "Phosphate Regeneration by Zooplankton in Cayuga Lake." *Limnol. and Oceanog.* 10. (1965).

109. Pomeroy, L.R., Mathews, H.M., and Min, H.S. "Excretion of Phosphate and Soluble Organic Phosphorus Compounds by Zooplankton." *Limnol and Oceanog.* *8*. (1963) 50-55.

110. Hayes, F.R. and Phillips, J.E. "Lake Water and Sediments. IV. Radiophosphorus Equilibrium with Mud, Plants, and Bacteria Under Oxidized and Reduced Conditions." *Limnol. and Oceanog.* *3*. (1958), 459-475.

111. Johannes, R.E. "Uptake and Release of Phosphorus by a Benthic Marine Amphipod." *Limnol. and Oceanog.* *9.* (1964), 235-242.

112. Johannes, R.E. "Influence of Marine Protozoa on Nutrient Regeneration." *Limnol. and Oceanog.* *10*. (1965).

113. Johannes, R.E. "Uptake and Release of Dissolved Organic Phosphorus by Representatives of a Coastal Marine Ecosystem." *Limnol. and Oceanog.* 9. (1964), 224-234.

114. Johannes, R.E. "Phosphorus Excretions and Body Size in Marine Animals: Microzooplankton and Nutrient Regeneration." *Science* *146*. (1964), 923-924.

115. Phillips, J.E. "The Ecological Role of Phosphorus in Waters With Special Reference to Microorganisms," In: *Principles and Applications in Aquatic Microbiology*, Edited by H. Henkelekian and N.C. Dendero, Pub. John Wiley and Sons, Inc. (1964), 61-81.

116. Shapiro, J. "Induced Rapid Release and Uptake of Phosphate by Microorganisms." *Science*. *155*. (March 1967), 1269-1271.

117. Fair, G.M. "Mathematical Aspects of Sewage Research." *Modern Sewage Disposal*. Ed. by Langdon Pense. Federation of Sewage Assoc. (1938).

118. Kratz, W.A. and Myers, J. "Nutrition and Growth of Several Blue-Green Algae." *Amer. J. Botany*. *42*. (1955), 282-287.

119. Barton, A.A. "Some Aspects of Cell Division in *Succharomyces cerevisiae*." *J. Gen. Microbiol*. *4*. (1950) 84-86.

120. Brook, T.D. *Principles of Microbial Ecology*. Published by Prentice-Hall, Inc. (1966).

121. Tomniaga, H. and Ichimara, S. "Ecological Studies on the Organic Production in River Ecosystem." *Botany Magazine of Tokyo*. 79. (1966), 815-829.

122. Paauw, F. Vander, "Der Einfluss der Temperatur aud Atmung und Kohlensaure Assimilation einger Grunalgen," *Planta*, *22*. (1934)

123. Phinney, H.K. and McIntire, C.D., "Effects of Temperature on Metabolism of Periphyton Communities Developed in Laboratory Streams, "*Limnol. & Oceanog*. *10*. (1965), 341-344).

124. Owens, M. "Some Factores Involved in the use of the Dissolved-Oxygen Distributions in Streams to Determine Productivity," *Mem. Ins. Ital*., *18 Suppl*. (1965), 209-224.

125. Fogg, G.E. *Algal Cultures and Phytoplankton Ecology*, Published by the University of Wisconsin Press, 1965.

126. Collyer, D.M. and Fogg, G.E. "Studies on Fat Accumulation by Algae." *J.Exp. Bot*. *6*. (1955), 256-275.

127. Lewin, R.A. "Extracellular Polysaccharides of Green Algae," *Canadian. J. of Microbiology*, *2*. (1956), 665-676.

128. Guillard, R.R.L. and Wangerksy, P.J. "The Production of Extracellular Carbohydrates by Some Marine Flagellates," *Limnol. and Oceanog*. *3*. (1958) 449-454.

129. Fogg, G.E. "Nitrogen Nutrition and Metabolism Patterns in Algae." *Symp. Soc. Exp. Biol*. *13*. (1959), 106-125.

130. Sorokin, C. "Changes in Photosynthetic Activity in the Course of Cell Development in *Chlorella*," *Physiol. Plant*. *10*. (1957), 659-666.

131. Ryther, J.H. "Inhibitory Effects of Phytoplankton upon the Feeding of *Daphnia Magna* with Reference to Growth, Reproduction and Survival." *Ecology*. *35* (4). (October, 1954), 522-533.

132. McMahon, J.W. and Rigler, F.H. "Feeding Rate of *Daphnia Magna Straus* in Different Foods Labeled with Radioactive Phosphorus." *Limnol. and Oceanog*. *10*. (1965), 105-113.

133. Stross, R.G., Unger, F.M., Jones, J.C., Vail, J.M. "Utilization of Algae by Daphnia as Influenced by Cell Senescence and UV Radiation." Proc. Industrial Waste Conf. Purdue University Eng. Extension Series No. 118. (1965).

134. Ketchum, B.H. "Mineral Nutrition of Phytoplankton." Annual Review of Plant Physiology. 5. (1954), 55-74.

135. Liebig, Justas. "Organic Chemistry in its Application to Vegetable Physiology and Agriculture," in Liebig's complete works on chemistry, Philadelphia, T.B. Peterson (1841).

136. Lund, J.W.G. "Studies on Asterionella Formosa hass, II. Nutrient Depletion and the Spring Maximum." J. of Ecology, 38. (1950), 1-35.

137. Hutchinson, G.E. "Limnological Studies in Connecticut. VLI. A Critical examination of the supposed relationship betwen phytoplankton periodicity and chemical changes in lake waters." Ecology, 25. (1944), 3-26.

138. Gerloff, G.C. and Skoog, F. "Nitrogen as a Limiting Factor for the Growth of Microcystis Aeroginosa in Southern Wisconsin Lakes." Ecology. 38. (4), (October, 1957), 556-561.

139. MacKenthum, K.M. Nitrogen and Phosphorus in Water. An Annotated Selected Bibliography of their Biological Effects, Publ. Health Service Publication of Washington, No. 1305. (1965).

140. Sawyer, G.M. "Basic Concepts of Eutrophication." Water Pollution Control Federation, I. 38. (5), (May, 1966), 737-744.

141. Richards, F.A., Cline, J.D., Broenkow, W.W. and Atkinson, L.P. "Some Consequences of the Decomposition of Organic Matter in Lake Nitinat, an Anoxic Fjord." Limnol. and Oceanog. 10. (1965).

142. Nesbitt, J.B. "Removal of Phosphorus from Municipal Sewage Plant Effluents." Pennsylvania State University Engineering Research Bulletin, B-93. (February, 1966).

143. Chu, S.P. "The Influence of the Mineral Composition of the Medium on the Growth of Planktonic Algae. Part I, Methods and Culture Media." Journal of Ecology, 31 (2). (1943), 109-148.

144. Chu, S.P. "The Influence of the Mineral Composition of the Medium on the Growth of Planktonic Algae. Part II, The Influence of the Concentration of Inorganic Nitrogen and Phosphate Phosphorus." Journal of Ecology, 31 (2), (1943), 109-148.

145. Rodhe, W. "Environmental Requirements of Fresh Water Plankton Algae." Symbolae Botanical Upsalienser. 10 (1), (1948), 1-149.

146. Gallaway, R.A. and Kraus, R.W. "Utilization of Phosphorus Sources by Chlorella," Microalgae and Photosynthetic Bacteria. (1963), 569-575.

147. Algal Culture from Laboratory to Pilot Plant, Edited by J.S. Burlew, Carnegie Institute of Washington Publication, #600. (1953).

148. Redfield, A.C., Ketchum, B.H., and Richards, F.A. "The Influence of Organisms on the Compositions of Sea Water," in The Sea, Chap. 2, Vol. 2. Edited by M.N. Hill, Interscience Publishers (1963).

149. Gerloff, G.C. and Krombholz, R.H. "Tissure Analysis as a Measure of Nutrient Availability for the Growth of Angiosperm Aquatic Plants." Limnol. and Oceanog. 11 (1966), 529-537.

150. Smillie, R.M. and Krotkov, G. "Phosphorus-Containing Compounds In Euglena Gracitis Grown under Different Conditions." Arch. Biochem. Biophys. 89. (1960), 83-90.

151. Ketchum, B.H. and Redfield, A.C. "Some Physical and Chemical Characteristics of Algal Growth in Mass Culture." J. Cell. Comp. Physiol. 33. (1949), 281-300.

152. Milner, H.W. "The Fatty Acids of Chlorella." J. of Biol. Chem. 176. (1948), 813-817.

153. Parsons, T.R., Stephens, K., and Skrickland, J.D.H. "On the Composition of Eleven Species of Marine Phytoplankters." Fish. Res. Bd. Canada Journal. 18. (1961), 1001-1016.

154. Strickland, J.D.H. "Measuring Production of Marine Phytoplankton." Published by the Fish Res. Bd. of Canada, Bulletin 122.

155. Spoehr, H.A. and Milner, H.W. "The Chemical Composition of Chlorella: Effect of Environmental Conditions." Plant Physiology. 24. (1959), 120-149.

156. Beerstecher, Jr., E. Petroleum Microbiology, Elseview Press. (1954).

157. VonKrevela, D.W. "Geochemistry of Coal." Organic Geochemistry. Edited by I.A. Breger, published by the MacMillan Co. (1965), 185-247.

158. Porges, N., Jaswiez, L. and Hoover, S.R. "Aerobic Treatment of Dairy Wastes." *Applied Microbiol*. *1*. (1953), 262.

159. McCarty, P.L. "Thermodynamics of Biological Synthesis and Growth." *Presented at the Second International Conference on Water Pollution Research*, Tokyo, Japan. (August, 1964).

160. Symons, J.M. and McKinney, R.E. "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge." *Sewage Works J*. *30* (7). (1958), 874-890.

161. Jenkins, D. and Menar, A.B. "The Fate of Phosphorus in Sewage Treatment Processes. 1. Primary Sedimentation and Activated Sludge." *SERL Report No. 67-6*, Univ. of Calif., Berkeley, Calif. (May, 1967).

162. Borchardt, J.A. and Azad, H.S. "Biological Extraction of Nutrients" *Paper presented at 40th Water Pollution Control Federation Conference*. New York. (October, 1967).

163. A.P.H.A. Committee on Sewage Disposal. "The Utilization of Sewage Sludge as Fertilizer." *Sewage Works Journal*, *9*. (1937), 861.

164. Anderson, M.S. "Comparative Analysis of Sewage Sludges." *Sew. and Ind. Wastes*. *28* (1956), 132.

165. Speece, R.E. and McCarty, P.L. "Nutrient Requirements and Biological Solids Accumulation in Anaerobic Digestion." *Proc. 1st. Inter. Conf. on Water Pollution Control*, London. (1962).

166. Sawyer, C.N. "Bacterial Nutrition and Synthesis." Biol. *Treatment of Sewage and Industrial Wastes*, *1*. Reinhold, New York. (1956), 3-17.

167. Eckenfelder, W.W. and Weston, R.F. "Kinetics of Biological Oxidization." *Biological Treatment of Sewage and Industrial Wastes*, *1*. Reinhold, New York. (1956), 18-24.

168. Thomas, W.H. and Krauss, R.W. "Nitrogen Metabolism in Scenedesmus as Affected by Environmental Changes." *Plant Physiology*, 30. (1955), 113-122.

169. Gerloff, G.C. and Skoog, F. "Cell Contents of Nitrogen and Phosphorus as a Measure of their Availability for Growth of Microcystis Aerogincsa." *Ecology*, *35* (3). (July, 1954), 348-353.

170. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Inc. (1966).

171. Wyckoff, B.M. "Rapid Solids Determination Using Glass Fiber Filters." *Water and Sewage Works*,(June, 1964).

172. El-Dib, M.A. and Ramadan, F.M. "Dichromate Versus Ceric Sulphate in COD Determinations." *Journal of the Sanitary Engineering Div. ASCE*, *92*, (SA2), Proc. Paper 4795. (April, 1966), 97-101.

173. Robert A. Taft Sanitary Engineering Center, *Water Oxygen Demand No. 2, Study Number 21*, Public Health Service Publication No. 999-WP-26. (1965).

174. Kirk, P.L. "Kjeldahl Method for Total Nitrogen." *Anal. Chem.*, 22. (1950), 354-358.

175. Markham, R. "A Steam Distillation Apparatus Suitable for Micro-Kjeldahl Analysis." *Biochem. J.*, *36*. (1942), 790-791.

176. Ruttner, F. *Fundamentals of Limnology*, Translated by D.G. Frey and F.E.J. Fry, University of Toronto Press. (1965).

177. Jenkins, D. and Medsker, LL. "A Method for the Determination of Nitrate-Nitrogen in Ocean, Estuarine and Fresh Waters." *Anal. Chem.*, *36*, (3). (1964), 610-612.

178. Kahn, L. and Brenenski, F.T. "Determination of Nitrate in Estuarine Water - Comparison of a Hydrazine Reduction and a Brucine Procedure and Modification of a Brucine Procedure." *Environmental Science and Technology*. (1967), 488-494.

179. Lee, G.F., Clesceri, N.L. and Fitzgerald, G.P. "Studies on the Analysis of Phosphates in Algal Cultures." *Int. J. Air Wat. Poll.* *9*. (1965), 715-722.

180. Loehr, R.C. and Rohlich, G.A. "A Wet Method for Grease Analysis." *Proc. XVII Industrial Waste Conference*, Purdue Engineering Extension Series Bulletin No. 112. (1962).

181. MacKinney, G. *J. Biol. Chem.*, *140*. (1941), 315-322.

182. Arnon, D.I. "Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris." *Plant Physiology*, *24*. (January, 1949), 1-14.

183. Smith, J.H.C. and Benitez, A. "Chlorophylls: Analysis in Plant Materials." In *Modern Methods of Plant Analysis, IV*. Edited by K. Paech and M/V. Tracey. Vol. IV. (1955). Pub. by Springer-Verlug - not commercially available.

184. Atkins, W.R.G. and Jenkins, P.G. "Seasonal Changes in the Phytoplankton during the year 1951-52 as Indicated by Spectrophotometric Chlorophyll Estimations." *J. Mar. Biol. Assoc. of U. K.*, *31*. (1953), 495.

185. Atkins, W.R.G. and Parke, M. "Seasonal Changes in the Phytoplankton as Indicated by Chlorophyll Estimations." *J. Mar. Biol. Assoc. of U.K.*, *29*. (1951), 609.

186. Skulberg, O.M. "Literature Concerning the Application of Algal Culture Experiments as a Means to Assess the Fertility of Water," (1965). *Personal Communication* received November 1, 1967.

187. Skulberg, O.M. "Algal Cultures as a Means to Assess the Fertilizing Influence of Pollution." *Third International Conference on Water Pollution Research*, Paper No. 6., Sec. 1, Munich, Germany. (1966).

188. Fitzgerald, G.P. Discussion in: "Environmental Requirements of Blue-Green Algae." *Proceedings of a Symposium*, Sponsored by the Univ. of Washington and the Federal Water Pollution Control Administration, U.S. Dept. of Interior. (October, 1967).

189. Tang, N.H. "Algal Growth Potential in Waste Water Effluents." Engineer's Thesis, Stanford University. (1966).

190. Rothstein, A. "The Enzymology of the Cell Surface." Protoplasmatologia Handbuch der Protoplasmaforschung II E4, *Springer-Verlag* Wien. (1954).

191. Brandes, D. and Elston, R.N. "An Electron Microscopical Study of the Histochemical Localization of Alkaline Phosphatase in the Cell Wall of *Chlorella vulgaris*." *Nature*, *177*. (1956), 274-276.

192. Bruce, H.E. and Hood, D.W. "Diurnal Inorganic Phosphate Variations in Texas Bays." *Publ. Inst. Mar. Sc: Texas*, *6*. (1959), 133-145.

193. Kuenzler, E.J. and Ketchum, B.H. "Rate of Uptake by Phacodactylum tricornutum." *Biological Bulletin*, *123*. (1962), 134-145.

194. Proctor, M.H. "Acid Phosphatase Activity of Some Algae and Its Inhibition by Molybdenum." *Biochem. Biophys. Acta*. *59*. (1962), 710-712.

195. Kuenzler, E.J. and Perras, J.P. "Phosphatases of Marine Algae." *Biological Bulletin*, *128*, (2). (April, 1965). 271-284.

196. Kuenzler, E.J. "Glucose-6-Phosphate Utilization by Marine Algae." *J. of Phycology*, *1*, (4). (1965), 156-164.

197. Karl-Kroupe, E., Callis, C.F., and Seifter, E. "Stability of Condensed Phosphates in Very Dilute Solutions." *Ind. Engng. Chem*. *49*. (1957), 2061-2062.

198. Englebrecht, R.S. and Morgan, J.J. "Studies on the Occurrence and Degradation of Condensed Phosphate in Surface Waters." Sewage and Industrial Wastes, 31, (4). (April, 1959), 458-478.

199. Eppley, R.W. "Hydrolysis of Polyphosphates by Porphyra and other Seaweeds." Physiol. Plant., 15. (1962), 246-251.

200. Clesceri, N.L. and Lee, G.F. "Hydrolysis of Condensed Phosphates i: Non-Sterile Environment." Int. J. Air. Wat. Poll., 9. (1965), 723-742.

201. Clesceri, N.L. and Lee, G.F. "Hydrolysis of Condensed Phosphates II: Sterile Environment." Int. J. Air Wat. Poll., 9. (1965), 743-751.

202. Shannon, J.E. and Lee, G.F. "Hydrolysis of Condensed Phosphates in Natural Waters." Int. J. Air Wat. Poll., 10. (1966), 735-756.

203. Davis, E.M. Degradation and Assimilation of Condensed Phosphates by Blue-Green and Green Algae. Ph.D. Dissertation, University of Oklahoma. (1966).

204. Chu, S.P. "The Utilization of Organic Phosphorus by Phytoplankton. J. Mar. Biol. Assoc. U. K., 26. (1946). 285-295.

205. Kuenzler, E. J. and Perras, J.P. "Separation of Dissolved Organic Phosphorus Compounds from Natural Sea Water." Woods Hole Oceanog. Inst. Report, Reference No. 65-59. (September, 1965), unpublished manuscript.

206. Riley, G.A. "Theory of Food-Chain Relations in the Ocean." In: The Sea, II (Chap. 20), Edited by M.N. Hill. (1963), 438-463.

207. Riley, G.A. "Plankton Studies. III. Long Island Sound." Bull. Bingham Oceanographic Collection, VII, (3). (1941).

208. Odum, H.T. "Primary Production in Flowing Waters." Limnol. and Oceanog. 1. (April, 1956). 102-117.

209. Odum, E.P. "Relationships Between Structure and Function in the Ecosystem." Japanese Journal of Ecology, 12. (1962), 108-118.

210. Odum, E.P. Ecology. Published by Holt, Rinehart and Winston, New York. (1963).

211. Edwards, C.A. and Heath, G.W. "The Role of Soil Animals in Break-down of Leaf Material. Soil Organisms." Edited by J. Doeksen and J. Van Der Drift, North Holland Publishing Co., Amsterdam.

212. MacKenthum, K.M., Herman, E.C. and Bartsch, A.F. "A Heavy Mortality of Fishes Resulting from the Decomposition of Algae in the Yahara River, Wisconsin." As published in: A Collection of Selected Papers on Stream Pollution, Waste, and Water Treatment, United States Dept. of the Int., FWPCA. (1967).

213. Young, J.C. and Clark, J.W. "Growth of Mixed Bacterial Populations at 20°C and 35°C." Water and Sewage Works. (July, 1965).

214. Potgieter, H.J. and Alexander, M. "Susceptibility and Resistance of Several Fungi to Microbial Lysis." J. Bacteriology, 91. (1966), 1529-1532.

215.Kuo, M.J. and Alexander, M. "Inhibition of Lysis of Fungi by Melanins." J. of Bact., 94. (1967), 624-629.

216 Kamen, M.D. Primary Processes in Photosynthesis. Published by Academic Press, New York. (1963).

217. Fogg, G.E. "Photosynthesis and Formation of Fats in a Diatom." Annals of Botany N.S., 20. (1956), 265-285.

218. Zobell, C.E. "The Effect of Solid Surfaces upon Bacterial Activity." J. Bact, 46. (1946), 39-56.

219. Plunkett, M.A. and Rakestraw, N.W. "Dissolved Organic Matter in the Sea." Deep Sea Research, Suppl. to Vol. 3. (1955), 12-14.

220. Parsons, T.R. and Strickland, J.D.H. "On the Production of Particulate Organic Carbon by Heterotrophic Processes in Sea Water." Deep Sea Research, 8. (1962), 211-222.

221. Rakestraw, N.W. "Oxygen Consumption in Sea Water over Long Periods." J. Mar. Res., 6. (1947), 259-263.

222. Vallentyne, J.R. "Solubility and the Decomposition of Organic Matter in Nature." Arch. Hydrobiol., 58., (4). (1962), 423-434.

223. Ketchum, B.H. and Corwin, N. "The Cycle of Phosphorus in Plankton Bloom in the Gulf of Maine." Limnol. and Oceanography, 10. (1965).

224. Brook, T.D. Principles of Microbiol Ecology. Published by Prentice-Hall, Inc. (1966).

225. Lewin, J.C. "Heterotrophy in Diatoms." J. Gen. Microbiol., 9. (1953), 305-313.

226. Lewin, J.C. and Lewin, R.A. "Auxotrophy and Heterotrophy in Marine Littoral Diatoms." Canad. J. Microbiol, 6. (1960), 127-134.

227. Sorokin, J.I. "On the Carbon Dioxide Uptake During the Cell Synthesis by Microorganisms." *Zeitschrift fur Allg. Mikrobiologie*, *6*, (1). (1966), 69-73.

228. Ryther, J.H. and Yentsch, C.S. "The Estimation of Phytoplankton Production in the Ocean from Chlorophyll and Light Data." *Limnol. and Oceanog.*, *2*. (1957), 281-287.

229. Stele, J.H. and Baird, I.E. "The Chlorophyll of Particulate Organic Matter in the Northern Sea." *Limnol. and Oceanog*, *10*. (1965).

230. Steeman Nielsen, E. and Jorgensen, E.G. "The Physiological Background for using Chlorophyll Measurements in Hydrobiologic and a Theory Explaining Daily Variations in Chlorophyll Concentrations." *Arch. Hydrobiol.*, *58*, (3). (1962), 349-357.

231. Bain, Jr., R.C. "Predicting Diurnal Variations in Dissolved Oxygen Caused by Algae in Estuarine Waters." *Proceedings of the National Symposium on Estuarine Pollution*, Stanford University. (August, 1967).

232. Lee, G.F., Clesceri, N.L., and Fitzgerald, G.P. "Studies on the Analysis of Phosphates in Algal Cultures." *Int. J. Air Water Poll.*, *9*. (1965), 715-722.

233. Harold, F.M. "Inorganic Polyphosphates in Biology: Structure, Metabolism, and Function." *Bacteriological Reviews*, *30*. (Dec. 1966), 772-794.